ENVIRONMENTAL FATE AND EFFECTS OF ATRAZINE, METOLACHLOR, CARBARYL AND CHLOROTHALONIL IN LOTIC ECOSYSTEMS

A DISSERTATION

SUBMITTED TO THE GRADUATE SCHOOL

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE

DOCTOR OF ENVIRONMENTAL SCIENCE

BY

DANIEL ELIAS

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BALL STATE UNIVERSITY

MUNCIE, INDIANA

MAY 2016

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ABSTRACT

TITLE: Environmental fate and effects of atrazine, metolachlor, carbaryl and chlorothalonil in lotic ecosystems

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Pesticides have the potential to affect receiving aquatic ecosystems. These effects are dependent on compound physicochemical characteristics, susceptibility of aquatic organisms, abundance in streams, and stream characteristics. Atrazine, metolachlor, carbaryl, and chlorothalonil have high usage rates in the U.S. and are detected in streams at concentrations that might have adverse effects on aquatic organisms. In this study, pesticide abundance and toxicity were quantified. Pesticide concentrations were differentially influenced by stream physicochemical parameters depending on the spatial scale. Further, pesticide concentration was influenced by compound octanol-water partition coefficient and solubility. Pesticides with higher affinity to water and specific modes of action (atrazine and carbaryl) did not affect sediment microbial nutrient uptake. In contrast, pesticides with higher affinity to sediments and broad modes of action (metolachlor and chlorothalonil) altered nutrient uptake. Pesticide effects were also measured on dominant grazing invertebrates, common freshwater snails. *Physa acuta* and *Helisoma anceps* egestion was lower with individual and combined pesticide exposure likely a result of narcosis and species-specific susceptibility. These data indicate that pesticide fate and abundance are influenced by compound characteristics and stream physicochemical properties and effects of pesticides on aquatic organisms are influenced by species characteristics.

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DEDICATION

There have been many people that have helped me along the way to complete this research. My advisor, Dr. Melody Bernot helped me become a better scientist, teacher, and team member. Mel, you showed me that hard work and dedication pays off. I am grateful of your mentorship as a scientist, educator, and your friendship. This dissertation is certainly dedicated to you.

To my family, this work is dedicated to you. My wife and sons, Miranda, Gabriel, and Julian, thank you for your patience, encouragement, and love. My mom and sister, Elia y Sarita, they always are extremely proud of me and have supported my doctorate journey. My dad, Luis, who taught me the scientific method and always makes me feel proud of my accomplishments. Finally, I dedicate this work to myself, the 6 year old me, who wanted to be a scientist, memorized dinosaur names, and mixed liquids to increase the growth of plants. We are a PhD little Daniel!

OVERVIEW

Crop protection activities including pesticide applications are increasing to fulfill the demand of a growing global human population for produce, fiber, medicine, and biofuels (Enserink et al. 2013). These agrochemicals have the potential to enter streams from many diffuse sources (nonpoint source pollutants) including rainfall, spray drift, and runoff (Cope, 1966). Atrazine, metolachlor, carbaryl, and chlorothalonil are detected in streams throughout the U.S. at concentrations that might reduce water quality and affect aquatic organisms (Larson et al. 1999). This dissertation was guided by two specific questions 1) What factors influence atrazine, metolachlor, carbaryl, and chlorothalonil abundance and fate in streams? and, 2) What are the effects of these pesticides on aquatic organisms?

In Chapter I, the factors that affect atrazine and metolachlor abundance in streams at both a local (Indiana) and national scale were determined. The dominant mechanisms driving atrazine and metolachlor concentrations across multiple scales were stream water quality parameters, crop selection (e.g., corn, soybean) and the agricultural practices associated with these crops (e.g., application rates, application schedule). In Chapter II, atrazine, metolachlor, and carbaryl transport in streams were quantified through direct measurement of uptake length, uptake velocity and areal uptake via short-term enrichment experiments. Pesticide transport was best predicted by compound solubility and pesticide concentrations.

Once pesticides enter freshwater ecosystems, lethal and sub-lethal effects on aquatic organisms may result which may alter ecosystem dynamics. Thus, the effects of atrazine, metolachlor, carbaryl, and chlorothalonil on sediment microbial community nutrient uptake were quantified (Chapter III). Sediment microbial nutrient uptake was affected by metolachlor and chlorothalonil, though no effects were measured with atrazine and carbaryl. These results were

consistent with hypotheses based on compound-specific modes of action and affinities. Pesticides with higher affinity to sediments affected nutrient uptake by decreasing ammonium and phosphate uptake (metolachlor) and decreasing nitrate remineralization and phosphate uptake (chlorothalonil).

In Chapter IV, the effects of individual and combined atrazine, metolachlor, carbaryl, and chlorothalonil on aquatic gastropods *Physa acuta* and *Helisoma anceps* egestion and movement were quantified. We observed species-specific response to pesticide exposure, with no effect for *H. anceps* and an 8-fold reduction in *P. acuta* egestion. Further, *H. anceps* movement declined with pesticide exposure though response varied with exposure time. Thus, in addition to pesticide compound characteristics, it is important to consider exposure duration to better understand the effects of pesticides on aquatic organisms.

Overall, this study highlights that water quality programs and risk assessments should incorporate local spraying schedules as well as compound solubility to enhance predictive models for targeted management of pesticide abundance and transport. Further, multiple organisms, pesticide mixtures, and exposure periods are needed to better assess response diversity and evaluate potential pesticide effect on freshwater ecosystems.

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CHAPTER I

SPATIAL TRENDS OF ATRAZINE AND METOLACHLOR ABUNDANCE IN STREAMS AT MULTIPLE SCALES

Abstract

Atrazine and metolachlor are detected in streams throughout the U.S. at concentrations that may have adverse effects on aquatic organisms and human health. Thus, there is a need to enhance predictive models of pesticide abundance across multiple scales. Stream samples were collected for quantification of pesticide and nutrient concentrations at eight sites in central Indiana for regional analysis of predictive factors. In addition, a meta-analysis of national data was conducted for comparison. Regionally, peak concentrations of atrazine and metolachlor did not occur during high discharge events, and there was no correlation between dissolved atrazine and metolachlor concentrations. However, sediment-bound metolachlor concentrations were positively related to sediment-bound atrazine at the regional scale. At the national scale, peak concentrations of atrazine and metolachlor were detected primarily in spring with the highest mean concentrations of atrazine in the Midwest region and metolachlor in the California region. At the national scale, dissolved atrazine concentrations were positively related to metolachlor concentrations and stream temperature but negatively related to pH. Further, dissolved metolachlor concentration was positively related to ammonium concentrations at the national scale. Pesticide use, application rates, and crop selection were dominant mechanisms driving atrazine and metolachlor concentrations at the national scale. At the regional scale, discharge was not the best predictor of observed pesticide concentrations due to drought conditions. Thus, in addition to discharge, timing of rainfall following application, and local spraying schedule should be considered to enhance predictive models for targeted management.

Introduction

Global human population growth and its concurrent demand for agricultural products (Grube et al. 2011) has increased the need for crop protection as well as use of pesticides for the control weeds, insects, and fungus (Enserink et al. 2013). In the United States, pesticide sales have increased ~10% since 1995 with 48% of these pesticides being herbicides, 35% insecticides, and 10% fungicides (Grube et al. 2011). This increased pesticide use has resulted in elevated pesticide concentrations in streams (Larson et al. 1999).

Pesticides are non-point source pollutants that enter freshwater habitats mainly via agricultural runoff, spray drift, and soil leaching (Cope, 1966; Larson et al. 1999). Once these pesticides are present in the aquatic ecosystem, they may have adverse effects on organisms (Relyea, 2006) including human health (Tanner et al. 2011). Several studies have reported direct mortality of diverse organisms such as tadpoles (Bridges, 2000; Relyea, 2006), fish (Munn et al. 2001; Napier et al. 2010), and benthic macroinvertebrates (e.g., amphipods and chironomids; Liess et al. 2005) with pesticide exposure. Further, at lower concentrations, sub-lethal effects can result in alterations in respiration rates (McMahon et al. 2012), growth (Relyea, 2006), and fecundity (Kashian et al. 2002) which may affect organismal fitness.

In the Midwestern U.S., atrazine and metolachlor have both high usage rates and prevalence in receiving waters (Larson et al. 1999; Gilliom et al. 2006). Atrazine is an herbicide used in the control of annual broadleaf and grass weeds predominantly in corn (Solomon et al. 1996). Metolachlor is an herbicide used in the control annual grasses and broadleaf weeds in soybean, cotton, peanuts, and potatoes (O'Connell et al. 1998). These pre-emergent herbicides are sprayed before weeds or crops emerge, usually in spring (Bauman and Ross, 1983).

These pesticides are measured in freshwaters at concentrations that may affect human health (Murray et al. 2010) and wildlife (Kohler and Triebskorn, 2013). USGS monitoring efforts at the national scale in the 1990s found that atrazine concentrations ranged from 2.4 - 201 µg/L (Larson et al. 1999) and metolachlor concentrations ranged from 1.2 - 77.6 µg/L (Kolpin et al. 1998; Larson et al. 1999) in the Midwestern U.S. At a concentration of 3 µg/L, atrazine increases amphibian susceptibility to trematode infection (Kiesecker, 2002; Kohler and Triebskorn, 2013). Further, atrazine concentrations of 0.11 µg/L have been linked to preterm births in humans when consumed in drinking water (Rinsky et al. 2012). Similarly, it has been suggested that metolachlor likely interferes with the perception of chemical stimuli by crayfish at 60 µg/L (Wolf and Moore, 2002; Cook and Moore, 2008) and reduces biomass and growth of aquatic plants at 43 µg/L (*Lemma gibba*; University of Hertfordshire, Pesticides Properties Database, 2014).

Though health advisory levels (HAL) and maximum contaminant level (MCL) concentrations have been established for atrazine and metolachlor (USEPA, 1995a; Nowell and Resek, 1994), pesticide regulation is limited by the spatial and temporal variation of atrazine and metolachlor concentrations in streams. Additionally, abundance of these pesticides is influenced by the local agricultural practices (Kreuger et al. 1999) and distinctive regional physicochemical conditions (Larson et al. 1999). For example, increasing alkalinity can lead to higher degradation rates of atrazine (Lartiges and Garrigues, 1995) and metolachlor (Kochany and Maguire, 1994). Further, pesticide concentrations in streams increase following rainfall, and the magnitude of this increase is influenced by the timing of rainfall with respect to the timing of pesticide application (Wauchope, 1978; Lerch and Blanchard 2003). However, atrazine base-flow concentrations have been negatively correlated with previous rainfall in August only (Kalhoff et al. 2003). Variability

in both pesticide concentrations as well as mechanisms driving concentrations yield predictive models of atrazine and metolachlor that are limited in their utility. Research must address the variable conditions that affect atrazine and metolachlor abundance in freshwaters in the context of specific environmental characteristics to better predict regional and national abundance and facilitate regulatory efforts.

Multiple studies have quantified atrazine and metolachlor concentrations in freshwater ecosystems (Larson et al. 1999; Gilliom et al. 2006; Smiley et al. 2014). However, limited syntheses (but see Capel et al. 2001; Larson et al. 2004; Stone et al. 2013) have been conducted to examine predictive variables at multiple spatial and temporal scales. For example, Watershed Regressions for Pesticides (WARP) in conjunction with the WARP for multiple pesticides (WARP-MP) were used to develop predictive models of atrazine abundance at the national scale (Larson and Gilliom, 2001). These models were subsequently updated (Larson et al. 2004; Stone et al. 2008; Stone and Gilliom, 2009; Stone and Gilliom, 2012) to include more explanatory variables and regional characteristics. However, these models do not successfully predict measured concentrations across variable sites and time periods (Stone et al. 2013). Thus, more research examining the relationships between stream pesticide concentrations and predictor variables is needed to enhance model development.

Understanding what factors affect atrazine and metolachlor concentrations in the context of spatial and temporal variability is a critical step needed to provide regulatory agencies with reliable predictive models to target management for aquatic and wildlife protection, remediation activities, and water quality assessment programs. To that aim, we conducted a regional assessment of atrazine and metolachlor in the agriculturally-intensive area of central Indiana. Additionally, we compared regional data to a national meta-analysis of available data to isolate

differences in regional- and national-scale patterns. We addressed the following specific research questions: 1) What stream physicochemical parameters affect atrazine and metolachlor concentrations at regional and national scales? and, 2) Do factors controlling atrazine and metolachlor concentrations vary with spatial scales?

Methods

Descriptive sampling

Eight streams were sampled monthly from October 2011 to September 2012 to quantify spatial and temporal variation in pesticide concentrations regionally (Table 1). Sites were located in the Upper White River Watershed (UWRW; Figure 1) of central Indiana with varied land use. The UWRW has a total area of 6993 km^2 with a gradient of both urban and agricultural land use. Killbuck Creek, Little Killbuck Creek, and Jakes Creek are located in the Killbuck Creek White River watershed which is predominantly agricultural (48.9% row crop, 11.9% herbaceous grasslands and pastures, 28.3% urban, 9.8% forest, and 0.4% wetland; White River Watershed Project, 2014). Duck Creek is located in the Duck Creek sub-watershed which is also dominated by agriculture (80.3% row crop, 11.3 urban, 4.3% herbaceous grass and pastures, and 4.0% forest). Weasel Creek, Prairie Creek, and Little Cicero Creek are part of the Cicero Creek agricultural watershed (71.9% in row crop, 7.7% herbaceous grass and pastures, 13% urban, and 5.5% forest). Little Eagle Creek is within the Eagle Creek watershed and is a mix of row crop (36.2%), urban (37.3%), herbaceous grass and pastures (14.2%), and forested (10.3%) land cover (White River Watershed Project, 2014).

Stream water was collected and analyzed for dissolved and sediment-bound concentrations of atrazine, metolachlor, nitrate (NO_3), phosphate (PO_4), chloride (Cl), sulfate (SO_4^2), bromide (Br),

ammonium (NH_4), and total organic carbon (TOC) as well as stream physicochemical characteristics (e.g., temperature, pH, conductivity, oxygen). For dissolved pesticide analyses, two filtered water samples (1000 mL) were collected at each site and filtered with a peristaltic pump (Geotech, Model 900-1280) through a 0.7 µm Whatman glass fiber filter into two amber baked glass bottles. These samples were delivered to the Indiana State Department of Health - Environmental Laboratories (Indianapolis, IN) for analysis of atrazine and metolachlor within 24 h of collection (Table 2). Atrazine and metolachlor in water samples were extracted from water by passing 1 L of sample water through a disk containing a solid matrix with a chemically bonded C18 organic phase (liquid-solid extraction, LSE). These compounds were eluted from the LSE disk with small quantities of ethyl acetate followed by methylene chloride. Atrazine and metolachlor were separated, identified, and measured by a gas chromatography/mass spectrometry (GC/MS) system (EPA method 525.2). For sediment-bound pesticide analyses, one composite homogenized sediment sample (top 5 cm) was collected by sub-sampling five points along a transect across the wetted width of each site. Coarse particulate matter was removed from the sediment. Collected sediment was then placed into a 40 mL glass vial which was subsequently delivered to the Indiana State Department of Health Chemical Laboratories within 24 h of collection (Table 2). For analyses of atrazine and metolachlor in sediments, samples were mixed with anhydrous sodium sulfate to form a free-flowing powder. The mixture was extracted with solvent three times, using ultrasonic extraction. The extract was separated from the sample by vacuum filtration or centrifugation (EPA method 525.2; Munch 1995) and then analyzed via GC/MS.

For cation and anion analyses, one filtered water sample (250 mL) was collected at each site. Nitrate (NO_3), phosphate (PO_4), chloride (Cl), sulfate (SO_4^2), and bromide (Br) were analyzed

using ion chromatography (DIONEX, ICS-3000). Ammonium (NH_4) was determined separately using the phenol-hypochlorite spectrophotometric procedure (APHA, 1995; Aminot et al. 1997). Samples for TOC analysis were acidified with 1.0 N HCl and measured using a Shimadzu TOC-L analyzer (Shimadzu Scientific Instruments, Columbia Maryland) using standard methods (Eaton et al. 2005). At each sampling site, stream physicochemical parameters were also measured in the thalweg using a Hydrolab minisonde for dissolved oxygen (LDO percentage saturation, LDO-mg/L), pH, temperature (T), total dissolved solids (TDS), specific conductivity (SpC), and salinity (Sal). A Watson-Marlow flow meter was used to estimate discharge with measurements of depth, width and velocity at multiple points along a cross-section of the channel.

Meta-analysis

Nationwide data on stream atrazine and metolachlor concentrations and physicochemical parameters were compiled from the U.S. Geological Survey national Water Quality Assessment (USGS NAWQA) data export page and from four peer-reviewed studies. Data collected from the USGS NAWQA included nine states: Washington (WA), Oregon (OR), Idaho (ID), California (CA), Indiana (IN), Illinois (IL), Ohio (OH), Wisconsin (WI), and Iowa (IA) from January 2000 to March 2014 (Figure 2).

The main criteria for data compilation was that the data available would have values of ammonia, nitrate, phosphate, pH, temperature, atrazine and metolachlor concentrations. Further, other group parameters were excluded from the search criteria (e.g., Biological, radiochemical, stable isotopes, microbiological, inorganic metals and non-metals, etc.). Overall, the data (N = 2348) included four search criteria parameters: “nutrients”, “organic pesticides”, “physical”, and

"information" of the available 16 parameter groups. Land use across sites was predominantly agricultural (70.3%), followed by mixed (12.8%), urban (4.5%), and forest (0.05%). Not available (NA) land use data occurred in 12.4% of data compiled.

For the selection of studies, we used search engines (i.e., Google Scholar and OneSearch). The search terms included "atrazine" and/or "metolachlor" in combination with "abundance", "prevalence", "usage", "detection frequency", "nutrients", "nitrate", "phosphate", "pH", and "temperature". These search terms provided >1000 results. We narrowed these results by selecting studies that provided a quantitative assessment of pesticide concentrations in U.S. freshwaters in conjunction with physicochemical parameters, and excluded studies conducted only in groundwater and that targeted pesticide metabolites (Final result N = 32). From this initial pool of studies, we contacted the authors for raw data, which narrowed our selected studies to four (Anderson et al. 1998; Kalhoff et al. 1998; Starner et al. 2003; Raffel et al. 2011). Anderson et al. 1998 sampled 16 sites in the Willamette River Basin (Oregon) twice in spring and fall of 1996 and analyzed 86 pesticides including atrazine and metolachlor. Quantification of atrazine (MDL: 0.001 µg/L) and metolachlor (MDL: 0.002 µg/L) followed USGS schedule 2010 (GC/MS) or USGS schedule 2051 (HPLC). Kalhoff et al. 1998 sampled 12 sites monthly in eastern Iowa from March to December of 1996 and measured chloroacetanilide herbicides. Metolachlor (Method Detection Limit, MDL: 0.05 µg/L) was analyzed by high-performance liquid chromatography according to the method of Meyer et al. 1993. Starner et al. 2003 sampled four sites in the San Joaquin River basin (California) from July 2nd to September 30th of 2002 and analyzed insecticides and herbicides. Atrazine (MDL: 0.02 µg/L) and metolachlor (0.02 µg/L) were measured using APCI/LC/MS/MS (Method EM38.0, Method 62.9; California Department of Pesticide Regulation, 2001). Raffel et al. 2011 sampled 18 sites in June, August, and

November 2010 and May 2011 to assess herbicides occurrence in central Indiana. Atrazine (MDL: 0.078 µg/L) and metolachlor (MDL: 0.09 µg/L) were measured using method EPA 525.2 (USEPA 1995a). Water quality data for these four studies were measured *in situ* at each sampling site using standard USGS guidelines.

Data analysis

SigmaPlot© 12.0 software was used for One way Repeated Measurements Analysis of Variance to analyze the effects of date of collection (N = 12) on pesticide concentrations followed by post-hoc Tukey tests to address differences between months. Spearman correlation was used to relate stream physicochemical characteristics to dissolved and sediment-bound pesticide concentrations regionally (UWRW, Central Indiana) and nationally (IN, IA, IL, OH, WI, WA, ID, OR, CA).

National dataset trends were initially assessed using Spearman correlation. However, after inspection, significant correlations were likely spurious and not biologically important. Thus, hierarchical linear mixed effect model fit with Bayesian inference (MCMCglmm package; Hadfield, 2010) was used to investigate the relationship between atrazine and metolachlor concentrations and stream ecosystem characteristics. Due to the complexity of the model (i.e., random slope and intercept), initial modeling attempts using the lmer() function of the lme4 package (Bates et al. 2014) and the lme() function of the nlme package (Pinheiro et al. 2015) and both failed to converge. Marcov Chain Monte Carlo methods are more efficient at finding a solution to complex problems where maximum likelihood methods fail or produce spurious results. Atrazine and metolachlor were modeled separately as a function of ammonium, nitrate,

phosphate, temperature, specific conductivity, and pH as fixed effects. Each model also included a fixed effect for the other pesticide measured. Thus, the model of atrazine was:

1. Model 1: $\text{atr}_i \sim \text{Normal}(\mu a_i, \sigma^2)$
2. Model 2: $\mu a_i = \alpha_{j[i]} + \beta_{1j[i]}(\text{ammonium})_i + \beta_{2j[i]}(\text{nitrate})_i + \beta_{3j[i]}(\text{phosphate})_i + \beta_{4j[i]}(\text{temperature})_i + \beta_{5j[i]}(\text{specific conductance})_i + \beta_{6j[i]}(\text{pH})_i + met\beta_{j[i]}(\text{metolachlor})_i$

Where atr_i is the concentration of atrazine at observation i, μa_i is the mean concentration of atrazine, σ^2 is the variance, α_j is the intercept for site j, $\beta_{1\text{-}6j}$ is the fixed effect of explanatory variables for site j, and $met\beta_j$ is the fixed effect of metolachlor. A hierarchical prior was used to represent random site effects for the intercept α_j, regression coefficients $\beta_{1\text{-}6j}$ and $met\beta_j$ where the j site level coefficients were drawn from an overall population level distribution. The overall population level distribution was given a non-informative normal prior with mean = 0 and variance = 1e+10 using default settings of the MCMCglmm function. The model of metolachlor was:

3. Model 3: $\text{met}_i \sim \text{Normal}(\mu m_i, \sigma^2)$
4. Model 4: $\mu m_i = \alpha_{j[i]} + \beta_{1j[i]}(\text{ammonium})_i + \beta_{2j[i]}(\text{nitrate})_i + \beta_{3j[i]}(\text{phosphate})_i + \beta_{4j[i]}(\text{temperature})_i + \beta_{5j[i]}(\text{specific conductance})_i + \beta_{6j[i]}(\text{pH})_i + atr\beta_{j[i]}(\text{atrazine})_i$

Where met_i is the concentration of atrazine at observation i, μm_i is the mean concentration of metolachlor, σ^2 is the variance, α_j is the intercept for site j, $\beta_{1\text{-}6j}$ is the fixed effect of explanatory variables for site j, and $atr\beta_j$ is the fixed effect of atrazine. A hierarchical prior was used to represent random site effects for the intercept α_j, regression coefficients $\beta_{1\text{-}6j}$ and $atr\beta_j$ where the j site level coefficients were drawn from an overall population level distribution. The overall

population level distribution was given a non-informative normal prior with mean = 0 and variance = 1e+10 using default settings of the MCMCglmm function.

Posterior distributions were estimated with three independent chains each with 15,000 iterations, 1,000 burn in steps, and thinned every third iteration. The Brooks-Gelman-Rubin statistics was used to evaluate if the mcm chain has reached a solution (convergence, posterior distribution) with values < 1.1 indicating convergence. Inference was made from the joint posterior distributions of population level parameters. Population level coefficients were considered significant when the 95% posterior credible interval did not overlap zero. Credible intervals are interpreted as a probabilistic statement about the true parameter. Thus, a 95% credible interval says there is a 95% probability the true parameter value falls between these bounds given the data, model, and priors. All analyses were conducted in R 3.1.1, unless otherwise stated (R Core Team, 2014).

Results

Regional assessment

Nutrients and physicochemical parameters

Nitrate (0.7 mg/L – 16.3 mg/L), PO_4 (< 0.01 mg/L – 7.1 mg/L) and TOC (4.2 mg/L – 122.6 mg/L) varied two orders of magnitude from October 2011 to September 2012 (Table 4). In contrast, NH_4 concentration (0.16 µg/L – 0.18 µg/L) varied less than one order of magnitude across sites and sampling events (Table 4). NO_3 (16.3 mg/L) and PO_4 (7.1 mg/L) peak concentrations were measured in February across all sampling sites. Total organic carbon peak concentration (122.6 mg/L) was measured in June. Ammonium peak concentration (0.18 µg/L) was measured in late summer and fall (Table 4). Conductivity (259 µS/cm – 680 µS/cm), salinity

(0.12 ppt – 0.35 ppt), TDS (0.16 g/L – 0.43 g/L), pH (7.37 – 8.3), and oxygen saturation (35.2% - 91.5%) varied less than one order of magnitude across all sites and sampling events although temperature (4.6 C° - 25.7 C°) and oxygen concentration (2.8 mg/L – 11.5 mg/L) varied one order of magnitude (Table 4). In contrast, discharge varied three orders of magnitude (6 L/s - 4102 L/s) across sampling events and sites (Table 4).

Spatial and temporal variation in pesticides

Atrazine and metolachlor concentration varied spatially across sampling sites. Dissolved atrazine and metolachlor were detected in all streams (Figure 3), sediment-bound atrazine and metolachlor were not detected in Duck Creek. Overall, peak concentrations of these pesticides did not occur during the highest discharge period (Jan, 2012; Figure 4A; Figure 4B; Table 4).

Dissolved metolachlor (66.67%), sediment atrazine (91.67%) and sediment metolachlor (83.3%) were detected 8 – 11x times more frequently than dissolved atrazine (8.3%) among sites. Further, peak concentration of atrazine detected in May 2012 was significantly different ($F_{11,70} = 10.741$, $p < 0.001$) than other months. Similarly, dissolved metolachlor peak concentration (October 2011) was significantly different than other months ($F_{11,70} = 4.08$, $p < 0.001$) excluding May, July, and August 2012, matching pesticide application schedules in Indiana. There was not a significant relationship between dissolved atrazine and dissolved metolachlor concentrations ($\rho = 0.41$; $p = 0.17$; Figure 5A). In contrast, dissolved atrazine was correlated to sediment-bound metolachlor ($\rho = 0.641$; $p = 0.02$). Sediment-bound atrazine was correlated to sediment metolachlor ($\rho = 0.65$; $p = 0.02$, Figure 5B) and TOC ($\rho = 0.65$; $p = 0.02$), and NH_4 ($\rho = -0.66$; $p = 0.02$). Sediment-bound metolachlor was correlated with TOC ($\rho = 0.83$; $p < 0.001$).

National assessment

Nutrients and physicochemical parameters

Ammonia (0.1 µg/L – 0.2 µg/L), PO_4 (0.1 mg/L – 0.6 mg/L), and pH (7 – 8.1) varied less than one order of magnitude from 2000 to 2014 across the selected studies. This variation was consistent with regional data. In contrast, temperature (10.9 C° – 16.7 C°) variation at the national scale was not consistent with the regional assessment due to geographical variation (Figure 2). Nitrate concentrations (1.8 mg/L – 13.3 mg/L) and conductivity (214 µS/cm – 1325 µS/cm) varied one order of magnitude for the same time period and locations (Table 5), consistent with regional data. Maximum concentration of nutrients were detected in Oregon (NH_3; 0.2 µg/L), Washington (NO_3; 13.3 mg/L), and California (PO_4; 0.6 mg/L). Stream temperature was highest in California sites (15.8 C°), specific conductivity was highest in Wisconsin sites (1325 µS/cm), and stream pH was highest in Indiana sites (8.1).

Spatial and temporal variation in pesticides

Peak concentrations of dissolved atrazine and metolachlor were detected in May (2000 – 2014) across the selected studies (Figure 6). Maximum concentration of atrazine was detected in Ohio (2.2 µg/L) and minimum concentration was detected in Idaho (0.007 µg/L). For metolachlor, maximum concentration was detected in California (14.75 µg/L), and the lowest concentration was detected in Idaho (0.002 µg/L; Table 5).

Atrazine was positively related to metolachlor (95% CI = 0.75 to 2.97), and negatively related to both pH (CI = -0.44 to -0.15), and temperature (95% CI = -0.44 to -0.15). Further, the effect size of temperature was smaller relative to metolachlor and pH (Figure 7A). In addition, metolachlor was positively related to ammonium (95% CI = 0.06 to 1.86; Figure 7B).

Discussion

Pesticide management strategies are primarily developed by scientists using data from national water quality assessment programs such as the U.S. Geological Survey's National Water Quality Assessment, National Stream Quality Accounting Network Programs, and Watershed Regressions for Pesticides for multiple pesticides (Larson et al. 1999; Larson et al. 2004; Stone et al. 2013). The objective of these pesticide management plans is to assist farmers, Natural Resources Conservation Services (NRCS), and local soil and water conservation districts. Further, these pesticides management strategies would improve their predictive accuracy by incorporating regional stream physicochemical parameters. Thus, with that purpose, we conducted a regional assessment of atrazine and metolachlor concentrations as well as stream water quality parameters in the agriculturally-intensive area of central Indiana, and a meta-analysis of national streams. Our study showed that atrazine and metolachlor followed similar abundance trends at both regional and national scales, and that these pesticide concentrations were influenced at different magnitudes by different parameters depending on the location (Indiana streams vs. National streams).

Variation of atrazine and metolachlor abundance is likely a result of local climate patterns and agricultural practices (e.g., pesticides applications). For example, peak concentrations of metolachlor were detected in October 2011 in Indiana (fall spraying). In contrast, at the national scale, peak concentrations of metolachlor were detected in May (2000 to 2014) likely due to increased discharge. Further, in 2011, atrazine and metolachlor usage in Indiana was 40X higher and 10X higher than atrazine and metolachlor usage in California, (USGS-PNSP, 2012). This suggests dissimilar spraying practices and precipitation regimens due to different agricultural management and climates across sampling sites.

Regionally, we observed a seasonal trend where peak concentrations of atrazine and metolachlor were not detected during periods of high discharge (January, 2012). Rather, peak concentrations of these pesticides were detected in October 2011 (metolachlor) and May 2012 (atrazine and metolachlor, Figure 4A), matching regional spraying schedule (Sharrat et al, 2003). While pesticide peak concentrations are typically positively associated with discharge as well as storm and flood events (Borah et al, 2003; Taghavi et al. 2010; Taghavi et al. 2011), management practices (e.g., timing of application) are an important factor in the timing of pesticide movement into streams (Borah et al, 2003; Sprague, 2005). Further, from April 2012 to September 2012, regional sites were classified as abnormally dry to drought-extreme (Rippey, 2012). Thus, atrazine and metolachlor peak concentrations measured monthly from 2011 to 2012 followed a seasonal pattern that was likely influenced by reduced precipitation, timing of rainfall following application, and local spraying schedule (Larson et al. 1999; Goolsby and Battaglin, 1993; Sharrat et al, 2003).

At the national scale, spatial variation in atrazine and metolachlor abundance is likely due to dissimilar pesticide application rate (Larson et al. 1999; Grube et al. 2011). For example, in the California region, where vegetables (16.2%), and fruits and nuts (41.9%) are the main crops (USDA – NASS, 2013), atrazine is sprayed at < 0.36 kg/km^2 and metolachlor application ranges from 0.44 to 5.5 kg/km^2. In contrast, in the Midwest (IN, IL, OH, WI, and IA), where corn (46.5%) and soybean (40.4%) are the dominant crops (USDA – NASS, 2013), atrazine and metolachlor application rates are > 14.85 kg/km^2 and > 5.46 kg/km^2, respectively (USGS – PNSP, 2012). Thus, atrazine and metolachlor concentrations in streams are influenced by higher pesticide application rates in corn and soybean fields (Midwest, USDA ERS 2015) relative to vegetables and produce fields (e.g., lettuce, tomatoes) where atrazine and metolachlor are not

frequently used. In addition, pesticide concentrations in freshwaters are dependent on crop selection and intensity (Environment Canada, 2011). For example, atrazine was detected more frequently in shallow groundwater influenced by wheat (60.4%) than peanut (9.8%) crops. Similarly, metolachlor detection frequency was higher in groundwater influenced by peanuts (26.2%) relative to wheat (9.3%) crops (Kolpin et al. 1998). Thus, at the national scale, spatial variation of atrazine and metolachlor concentrations are also influenced by both crop selection (Gilliom et al. 2006) and the recommended crop application rates for atrazine (corn) and metolachlor (soybean, vegetables; USEPA, 1995b; USEPA, 2003).

The relationship between atrazine and metolachlor concentrations with stream physicochemical characteristics was influenced by the spatial scale. For example, in Indiana streams, dissolved metolachlor was negatively related with phosphate and oxygen concentrations, and positively related with sediment-bound atrazine. Further, sediment-bound metolachlor was positively correlated with TOC and sediment-bound atrazine. In the national assessment, atrazine concentration was positively related to temperature, and negatively related to pH (Figure 7A); while metolachlor was positively related to NH_4 (Figure 7B). Further, the importance of stream physicochemical conditions in predicting atrazine and/or metolachlor relationships with stream parameters is also influenced by variable data collection methods (i.e., sampling frequencies, Stone et al. 2013), availability of stream data for watersheds (Larson et al. 2004), and range of stream parameters associated with spatial scale (Temnerud and Bishop, 2005).

In our study, across a wider range of both dissolved atrazine and metolachlor concentrations (national scale, Table 5), atrazine concentration was positively related to metolachlor (Figure 7A & B) and increased with increasing metolachlor for all states but California (Figure 8). In

contrast, across a more narrow range of pesticides (Indiana; Table 4), there was not a relationship between atrazine and metolachlor concentrations (Figure 5A). Thus, the relationship between atrazine and metolachlor was likely affected by regional agricultural practices including pesticide application rates and state crop selection (Thurman et al. 1991; Smiley et al. 2014). In addition, sediment-bound atrazine was correlated to sediment-bound metolachlor (Figure 5B) at the regional scale; possibly, atrazine and metolachlor concentrations followed a similar trend (Figure 4B) due to simultaneous runoff and historical accumulation of these pesticides in stream sediments (Nodler et al. 2013).

In this meta-analysis, atrazine and metolachlor abundance is influenced by spraying schedules and crop selection. These factors contribute differently to pesticide concentrations in streams depending on rainfall events at both the regional and national scale. Further, predictive models of pesticide abundance in freshwater ecosystems incorporate different stream parameters depending on the spatial scale. For atrazine and metolachlor management, it is necessary to consider local stream parameters to fine tune predictive models developed at the national scale. Further, the development of region-specific models would provide regulatory agencies with more precise predictions of pesticide abundance in freshwater habitats to assist wildlife protection, remediation activities, water quality programs, preliminary analyses and screening level assessments.

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CHAPTER II

PESTICIDE TRANSPORT IN AN AGRICULTURALLY INFLUENCED STREAM IN INDIANA

Abstract

Agrochemicals, including pesticides and fertilizers, can be transported from agricultural fields into streams where they might have adverse effects on water quality and ecosystems. We conducted three enrichment experiments in a central Indiana agricultural stream to quantify pesticide and nitrogen transport dynamics. Specifically, we assessed transport of nitrate, atrazine, metolachlor, and carbaryl through direct measurement of uptake length (S_w), uptake velocity (V_f), and areal uptake (U). Overall, S_w varied less than one order of magnitude across pesticides with the highest S_w for atrazine suggesting greater transport to downstream ecosystems. Across compounds, pesticide S_w was longest in August relative to October and July. V_f varied less than one order of magnitude across pesticide compounds with highest V_f for metolachlor. U varied three orders of magnitude across pesticides with the highest U associate with sediment-bound carbaryl. Increasing nitrate S_w, with corresponding decreasing demand of nitrate, suggests that the supply of nitrate exceeds the demand of aquatic organisms in this central Indiana stream. Similarly, pesticide uptake metrics were dictated by pesticide concentrations. Overall, pesticide transport was best predicted by compound solubility which can complement and improve current models of pesticide abundance used by water quality programs and risk assessments.

Introduction

World population is expected to surpass 9 billion by 2100 (Gerland et al. 2014) from the current 7.2 billion (U.S. Census Bureau, 2014) with a concurrent need for increased crop production. Thus, agricultural activities (e.g., crop protection, fertilizer and pesticide application) are increasing in response to global population growth coinciding with higher pesticide use worldwide (Enserink et al. 2013). In the U.S., pesticide sales have increased ~10% in the last decade (Grube et al. 2011). The continued demand for agricultural products, and corresponding increasing fertilizer and pesticide usage, has resulted in higher occurrence and prevalence of nutrients (Jordan et al. 1997; Bernot et al. 2006) and pesticides in freshwaters (Larson et al. 1999, Toccalino et al. 2014). These agriculturally derived contaminants have the potential to cause eutrophication (Conley et al. 2009), decrease biodiversity (Bobbink et al. 2010) and affect human health (Fewtrell, 2004).

In the U.S., nitrogen, specifically nitrate (NO_3), is often detected in streams influenced by agricultural or urban settings with highest concentration in the Midwest, Northeast, California and Northwest U.S. (Dubrovsky et al. 2010). Nitrate readily dissolves in water and has the potential for moving in both streams and groundwater (Wilson et al. 2010). Nitrate has been detected at concentrations that have an adverse effect on human health. Nitrate at concentrations > 10 mg/L in drinking water can cause blue baby syndrome in infants (methemoglobinemia; Knobeloch et al. 2000). In natural ecosystems, increasing concentrations of NO_3 result in eutrophication, algal blooms, hypoxic zones, and fish kills (Conley et al. 2009), as well as higher rates of sediment nitrification (Duff et al. 2009).

Movement of nitrogen in lotic ecosystems is frequently measured using short-term enrichment studies (Tank et al. 2006). In general, a concentrated solution is dripped continuously into a

stream, in conjunction with a conservative tracer, until equilibrium is achieved (stable concentration). Once plateau is reached, water and sediment samples are collected downstream of the enrichment location. Differences in enriched compound concentrations, relative to the conservative tracer, are then used to calculate transport metrics. Using this enrichment method, uptake length (S_w), uptake velocity (V_f) and areal uptake (U) values can be calculated. Uptake length measures the distance traveled by a nutrient along the stream reach and represents the nutrient use efficiency in streams (Mulholland et al. 2002; Bernot et al. 2006; Tank et al. 2006). Uptake velocity measures the velocity a nutrient moves (biotic demand) from the water column (nutrient concentration) toward the benthos (Hall and Tank, 2003). Areal uptake represents the amount of nutrient immobilized by the biota in an area of streambed per unit of time (Tank et al. 2006). These uptake metrics are useful for comparing stream nutrient cycling across spatial and temporal scales (Mulholland et al. 2002) in the context of variable climate (Mulholland et al. 2008), biotic activity (Bernot et al. 2006), and management (Roley et al. 2012).

Atrazine, metolachlor (herbicides), and carbaryl (insecticide) have been detected dissolved and sorbed to sediments in Indiana streams, as well as throughout the Midwestern U.S. (Larson et al. 1999, Gilliom et al. 2007; Smiley et al. 2014). Atrazine and metolachlor are mainly detected dissolved in water (Bacci et al. 89) due to their higher solubility (Battaglin et al. 2002; Stackelberg et al. 2004). Carbaryl is most frequently detected dissolved in streams (Gilliom, 2007, Gunasekara et al. 2008). However, carbaryl is also detected sorbed to stream sediments (Daniels et al. 2000). These pesticides are detected in freshwater ecosystems at concentrations that may have adverse effects on aquatic organisms. For example, atrazine exposure has been linked to demasculinized frogs (Hayes et al. 2001), metolachlor can reduce nutrient assimilation of sediment microbial communities (Elias and Bernot, 2014), and carbaryl can decrease survival

of stream invertebrates (Peterson et al. 2001). Though pesticide concentrations are well documented (Larson et al. 1999; Gilliom et al. 2007), pesticide movement within lotic ecosystems is not well understood.

Many factors dictate compound movement and assimilation within stream ecosystems. Transport dynamics are typically correlated with compound concentrations (Newbold et al. 1982), with compounds having higher concentrations travelling further before removal from the water column (Webster et al. 2003). Physicochemical properties of a compound, including octanol-water partition coefficient (K_{ow}) and solubility (Neumann et al. 2002; Kuivila and Foe 2009) also influence compound movement in lotic ecosystems. For example, chemicals with higher solubility (e.g., Aldicarb, Dicamba) will remain in water and likely not accumulate in sediments or biota (Linde 1994; National Research Council, 2014). Generally, compounds with a high K_{ow} (e.g., chlorothalonil: 2.9; DDT: 6.11) will not remain in the water column but likely sorb to sediments and accumulate in organisms (Linde, 1994). In natural ecosystems, the interactions of compound properties (K_{ow}, solubility) with abundance and the biotic community might influence the persistence of agricultural contaminants in the environment (Katagi, 2006). Several studies have assessed *in vitro* fate of atrazine, metolachlor, and carbaryl (Nowell et al. 2010; Balci et al. 2009; Gamble, 2009; Fenner et al. 2013). Additionally, indirect measurements of pesticide transport have followed the movement of pesticides applied to fields into the surrounding watersheds (e.g., Davis et al. 1993, Kruger et al. 1996; Kolpin et al. 1998; Larson et al. 1999, Sullivan et al. 2009). However, few studies have *directly quantified in situ* contaminant transport and retention in streams, limiting our predictive ability. Thus, direct measures of contaminant transport are needed to enhance our understanding of pesticide movement in streams and to increase the precision of previously developed pesticides transport models using

indirect measurements. We address this gap in knowledge by targeting two hypotheses: 1) Pesticide movement in stream increases at higher concentration of pesticides; 2) Pesticide transport is better predicted by compounds octanol-water partition coefficient and transport decreases with increasing K_{ow} (metolachlor > atrazine > carbaryl).

Methods

Study Site

Enrichment experiments were conducted at Jakes Creek (40.234494, -85.452059) on July 21st and October 18th in 2011 and August 5th in 2013. Jakes Creek runs east to west along the northern boundary of Cooper Field Station (Ball State University, Muncie, IN). Jakes Creek is surrounded by approximately 0.07 km^2 of forest and 0.06 km^2 of secondary succession. Jakes Creek is located in the Killbuck Creek White River watershed (HUC 0512020103) which is predominantly agricultural (48.9% row crop, 11.9% herbaceous grasslands and pastures, 28.3% urban, 9.8% forest, and 0.4% wetland; White River Watershed Project, 2014). Killbuck Creek White River watershed is part of the Upper White River Watershed (UWRW) of central Indiana. The UWRW has a total area of 6992.9 km^2 with a gradient of both urban and agricultural land use.

Experimental stream enrichment

Sampling stations (0 - 10) along the stream were established every 50 m along a 500 m reach (N = 10 sampling stations). Station 0 was located below a rock filled dam, under full sun with a riparian zone transitioning from tall grass into large trees and shrubs. Station 0 was dominated by cobble and sand substrate. Stations 1 through 10 were surrounded by large trees (e.g., oaks, maples, white ash, elm, sycamore). These stations received partial to no sun exposure throughout

the day. Substrate was a mix of silt and sand. At Jakes Creek, there were two visible tile drainages at station 0 and at station 5. During the October 2011 and August 2013 enrichment events, atrazine, metolachlor, carbaryl, and nitrate were dripped into the stream at station 0 to enrich stream concentrations (see below). For the July 2011 enrichment, only carbaryl, and nitrate were enriched.

Station 0 corresponded to the enrichment location and a reference station was located ~10 m upstream the enrichment to quantify background conditions. At station 0, A 25 L carboy was prepared with a mixture of the target commercial grade pesticides: atrazine (Atrazine 4L, 42.2% purity, Loveland, CO, US), metolachlor (Me-too-lachlor II, 84.4% purity, Drexel Chemical Company, TN, US), and carbaryl (Sevin XLR Plus, 44.1% purity, Bayer, NC, US). Pesticides (689.8 µg/L of atrazine, 176.8 µg/L of metolachlor, and 3.45 µg/L of carbaryl) were added to the carboy to reach target concentrations in the stream of 2.0 µg/L of atrazine, 1.0 µg/L of metolachlor, and 0.01 µg/L of carbaryl. Stream target enrichment concentrations were selected to represent environmentally relevant concentrations of these pesticides detected throughout the U.S. Nitrate was also added to the carboy as potassium nitrate (2397.3 g) and bromide (213.4 g) was included as a conservative tracer to achieve target stream concentrations of 0.2 mg/L and 1 mg/L, respectively. Pesticide, nitrate, and bromide added to the carboy varied with each event to account for differential stream discharge (Jakes Creek: 8 L/s; 2013: 34.7 L/s, White River; Figure 9) and maintain target enrichment concentrations.

Once the carboy solution was prepared, the solution was dripped into the stream reach continuously for two hours using a peristaltic pump (Watson-Marlow® Alitea) at 17.4 mL/min. Preliminary conservative tracer enrichments (bromide) were conducted to estimate duration of enrichment necessary to reach equilibrium (stable concentrations) at the most downstream

station (station 10). The carboy solution was dripped in an area of high mixing to ensure equal distribution of the enrichment solution. After ~120 min of enrichment, when downstream concentrations equilibrated (i.e., reach plateau), water and sediment samples were collected at each downstream sampling station, as well as the upstream reference station, for measurement of atrazine, metolachlor, carbaryl, and nitrate concentrations. For pesticide analyses, two filtered water samples (1000 mL) were collected at each sampling station and filtered with a peristaltic pump (GeoTech, Model 900-1280) through a 0.7 μm pore Whatman glass fiber filter (GF/F) into two amber baked glass bottles. For sediment-bound pesticide analyses, one composite homogenized sediment sample (top 5 cm) was collected by sub-sampling five points along the wetted width at each sampling station. Coarse particulate matter was removed from the sediment. Collected sediment was then placed into a 40 mL glass vial. Water and sediment samples for pesticide analyses were delivered to the Indiana State Department of Health – Environmental Laboratories (Indianapolis, IN) within 24 h of collection.

Atrazine and metolachlor in water samples were analyzed following EPA method 525.2 (Table 6). These pesticides were extracted from water by passing 1 L of sample water through a disk containing a solid matrix with a chemically bonded C18 organic phase (liquid-solid extraction, LSE). These compounds were eluted from the LSE disk with small quantities of ethyl acetate followed by methylene chloride. The sample components were separated, identified, and measured by gas chromatography/mass spectrometry (GC/MS). Carbaryl was analyzed following EPA method 531.1 (Table 6). Carbaryl was injected into a reverse phase HPLC column. Separation of carbaryl was achieved using gradient elution chromatography. After elution, carbaryl was hydrolyzed with 0.05N sodium hydroxide at 100°C. The methyl amine formed

during hydrolysis was reacted with o-phthalaldehyde (OPA) and thiofluor to form a highly fluorescent derivative which was detected by a fluorescence detector.

For analyses of atrazine and metolachlor in sediments (Table 6), samples were mixed with anhydrous sodium sulfate to form a free-flowing powder. The mixture was extracted with solvent three times, using ultrasonic extraction. The extract was separated from the sample by vacuum filtration or centrifugation. This extract was quantified for atrazine and metolachlor concentration following EPA method 525.2 (Munch, 1995). For analysis of carbaryl in sediments, samples quantification followed EPA methods 3550C (USEPA, 2007) and 531.1 (Graves, 1989). Samples were mixed with anhydrous sodium sulfate and extracted with solvent three times using ultrasonic extraction. This extract was separated from the sample by vacuum filtration or centrifugation. The extract was then measured for carbaryl concentration via GC/MS

For cation and anion analyses, one filtered water sample (250 mL) was collected at each station. Nitrate, phosphate, and bromide were analyzed using ion chromatography (DIONEX, ICS-3000). Ammonium was determined separately using the phenol-hypochlorite spectrophotometric procedure (APHA, 1995; Aminot et al. 1997). At each sampling station, stream physicochemical parameters were also measured in the thalweg using a Hydrolab minisonde for dissolved oxygen, pH, temperature, total dissolved solids, specific conductivity, and salinity. A Watson-Marlow flow meter was used to estimate discharge with measurements of depth, width and velocity at multiple points along a cross-section of the channel.

Data analysis

At each enrichment event (N = 3), transport metrics were calculated. Uptake length (S_w) was measured to calculate pesticide movement downstream before degradation or removal from

water column. A linear regression of the natural logarithm of the ratio of compound concentration relative to bromide concentration versus distance downstream from release site was used to calculate the decay rate (Figure 10):

$$\frac{\ln\left(\frac{compound}{Bromide}\right)}{distance} \quad \ldots\ldots\ldots\ldots (1)$$

Uptake length was calculated as the inverse of the decay rate (*k*):

$$S_w(m) = k^{-1} \quad \ldots\ldots\ldots\ldots (2)$$

Uptake velocity (V_f) is the velocity at which the pesticide or nitrogen is removed from the water column. Uptake velocity was calculated as:

$$V_f(m/min) = Qk/w \quad \ldots\ldots\ldots\ldots (3)$$

Where Q is discharge (m^3/min), w = mean stream wetted width. Areal uptake (U) is the amount of pesticide or nitrogen change from the water column to the biota (Tank et al. 2006). Areal uptake was calculated from the N_b (background N) and V_f:

$$U = V_f N_b \quad \ldots\ldots\ldots\ldots (4)$$

To calculate uptake metrics for pesticide concentrations below detection limit (BDL), we used half of the BDL value (Croghan et al. 2003). One-way analysis of variance was used to compare transport metrics among enrichment experiments as well as compound types. Differences between treatment concentrations among enrichment events were assessed with Tukey's multiple comparison tests. If normality assumption was violated, Kruskal-Wallis one-way analysis of variance on ranks was performed. Analyses were conducted using SigmaPlot©

12.0 software. Uptake metrics were subsequently compared to a nationwide assessment (Mulholland et al. 2008), employing similar enrichment techniques, for comparative assessment.

Results

Physicochemical variation

Stream discharge (L/s), dissolved oxygen (%), and pH differed among enrichment events (Table 7). Specifically, in August discharge (35.1 L/s) was 6x higher than October (6.0 L/s) and 4x higher than July (8.1 L/s; H = 17.57, d.f. = 2, p = < 0.001). Dissolved oxygen was also higher in August (65.0 %) relative to October (51.1 %) and July (54.2 %; $F_{(2,29)} = 5.795$; p = 0.008). In August, pH (7.7) was 3% higher than October (7.5) and 12% higher than July (6.8; $F_{(2,29)} = 64.128$; $p < 0.001$). Stream temperature, specific conductivity, salinity, nitrate background concentration, and total dissolved solids did not differ among enrichment events ($p > 0.05$).

Nutrient uptake

Nitrate uptake length (S_w) was the longest in August; specifically, July (23.6 m) was 3x shorter than October (81.3 m) and 28x shorter than August (666.7 m; Figure 11). In contrast, Uptake velocity (V_f) was the greatest in July; specifically, July (0.14 cm/min) was 7x higher than October (0.02 cm/min) and 8x higher than August (0.017 cm/min; Figure 11). Similarly, areal uptake (U) was the highest in July (0.038 mg N/cm^2/min); and 10x higher than October (0.004 mg N/cm^2/min) and 38x higher than August (0.001 mg N/cm^2/min) enrichments (Figure 11).

Pesticide concentrations

Background concentrations of atrazine and metolachlor were highest in August, followed by July and October. Dissolved concentration of atrazine in August (3.1 µg/L) was 3x higher than July (1.1 µg/L) and 22x higher than October (0.14 µg/L). Dissolved concentration of metolachlor was 6x higher in August (0.56 µg/L) than both July and October (0.09 µg/L). Dissolved concentrations of carbaryl for July, August, and October as well as sediment-bound carbaryl concentrations were below detection limit.

Pesticide transport

Uptake length (S_w) and uptake velocity (V_f) varied less than one order of magnitude across pesticides (Table 8). Specifically, atrazine uptake length (mean = 666m) was 5x higher than carbaryl (mean = 146.7 m), and 1.4x higher than metolachlor (mean = 469.3 m). Similarly, atrazine uptake velocity (mean = 0.015 cm/min) was 3x slower than carbaryl (mean = 0.045 cm/min), and 2x lower than metolachlor (mean = 0.037 cm/min). In contrast, areal uptake varied three orders of magnitude across pesticides (Table 8), where atrazine areal uptake (mean = 0.0165 µg atrazine/cm^2/min) was 14x lower than carbaryl (mean = 0.236 µg atrazine/cm^2/min), and 2.5x higher than metolachlor (mean = 0.0065 µg metolachlor/cm^2/min).

Atrazine uptake metrics varied 1 to 2 orders of magnitude across enrichment events (Figure 12). Atrazine uptake length in October (82 m) was 15x shorter than August (1250 m). In contrast, atrazine uptake velocity in October (0.02 cm/min) was 2x higher than August (0.009 cm/min). Further, atrazine areal uptake in October (0.003 µg atrazine/cm^2/min) was 10x lower than August (0.03 µg atrazine/cm^2/min). Metolachlor uptake length in October (29.6 m) was 31x lower than August (909 m; Figure 12). In contrast, metolachlor uptake velocity in October (0.06

cm/min) was 5x higher than August (0.013 cm/min). Areal uptake of metolachlor in October (0.006 μg metolachlor/cm^2/min) was 1.1x lower than August (0.007 μg metolachlor/cm^2/min). Carbaryl transport varied less than one order of magnitude across enrichment events (Figure 12). Carbaryl uptake length in July (100 m) was 1.9x lower than August (192.3 m). Carbaryl uptake velocity in July (0.03 cm/min) was 2x slower than August (0.06 cm/min). In contrast, areal uptake of carbaryl in July (0.35 mg carbaryl/cm^2/min) was 3x higher than August (0.12 μg carbaryl/cm^2/min). Sediment-bound carbaryl uptake metrics varied from ~2 orders of magnitude across enrichment events (Figure 13). Uptake length of sediment-bound carbaryl in July (192 m) was 8x higher than October (25.5 m). In contrast, uptake velocity of sediment-bound carbaryl in July (0.02 cm/min) was 3.5x lower than October (0.07 cm/min). Similarly, areal uptake of sediment-bound carbaryl (0.034 μg carbaryl /cm^2/min) was 42x lower than October (1.43 μg carbaryl/cm^2/min).

Discussion

Previous research has focused primarily on pesticide environmental fate using ***in vitro*** mesocosms as well as direct quantification of atrazine, metolachlor, and carbaryl concentrations (Larson et al. 1999; Gilliom et al. 2007). Our research is the first to our knowledge to quantify transport of atrazine, metolachlor, and carbaryl along the stream reach via **direct** and ***in situ*** measurements using enrichment techniques. We observed variation in nitrate, atrazine, metolachlor, and carbaryl concentrations as well as uptake metrics likely influenced by agricultural management and compound physicochemical characteristics. These results support our hypothesis of greater pesticide movement with higher pesticide concentration. However, our results do not support decreased pesticide transport with increasing octanol water partition

coefficient. Instead, compound solubility better predicted pesticide movement with greater pesticide transport associated with higher pesticide solubility (carbaryl > atrazine > metolachlor).

Pesticide uptake dynamics in streams are affected by pesticide concentrations, which is affected by timing of pesticide applications (Kuivila and Foe, 1995) and pesticide usage (Johnson et al. 2010). We observed pesticide concentrations and their corresponding uptake metrics to vary across enrichment events (Table 8); likely a result of pesticide runoff (Schulz, 2004) after local application periods (Wauchope 1978). Further, pesticide transport in streams is influenced by pesticide physical and chemical properties (Linde, 1994; Gavrilescu, 2005; Wijekoon et al. 2013). The octanol-water partition coefficient (K_{ow}) and solubility are broadly used to determine compound affinity to water or solids (Kawamoto and Urano 1989; Wauchope et al. 1992; Sabljic, 2001; Nowell et al. 2010). For example, pesticides with a log $K_{ow} < 2.7$ (atrazine and carbaryl) are likely detected in to sediments (Wauchope et al. 2002). Further, pesticides with high solubility will be detected more frequently dissolved in the water column relative to adsorb to sediments (Linde, 1994).

Though the octanol-water partition coefficient is broadly used to determine pesticides occurrence in streams (Gustafson, 1989; Finizio et al. 1997), in our study, pesticide solubility was a better predictor of atrazine, metolachlor, and carbaryl occurrence and transport than the octanol-water partition coefficient (Figure 14). Pesticides with lower solubility (carbaryl: 9.1 mg/L) were detected in sediments; whereas, water-soluble pesticides (atrazine: 35 mg/L and metolachlor: 530 mg/L) were detected only in the water column. Further, atrazine and metolachlor traveled further along the stream reach (mean Sw: 666 m, mean Sw: 469 m) than the less soluble carbaryl (mean Sw: 148 m). This suggests that less soluble compounds are bound to

particles (e.g., sediments, organic matter; Yang et al. 2015), which reduces pesticide transport in streams but increases deposition and adsorption to sediments (Li et al. 2015).

Similar to pesticides, nitrogen concentrations and nitrate uptake metrics are also influenced by spatial scale (Mulholland et al. 2008; Stone et al. 2013). Thus, we compared nitrate uptake kinetics in this study (Jakes Creek - Muncie, IN) with a nationwide study of 23 agricultural streams across the U.S. and Puerto Rico (Mulholland et al. 2008). We observed dissimilar nitrate uptake metrics in Jakes Creek relative to the national scale, likely influenced by location (Mulholland et al. 2008) and nitrate concentrations (Dodds et al. 2002; Bernot and Dodds, 2005). Specifically, higher nitrate concentrations nationwide stimulated higher nitrate uptake rate per area of streambed (U) relative to Jakes Creek. Similarly, uptake length at the national scale was one order of magnitude greater than Jakes Creek. In contrast, uptake velocity was two orders of magnitude lower than Jakes Creek. These uptake metrics suggest saturated conditions at the national scale because of reduced efficiency at removing nitrate relative to Jakes Creek. Overall, nitrate uptake metrics are likely also a function of variable stream conditions including light and temperature (Gregory, 1980) as well as different autotrophic and heterotrophic communities (Grimm, 1987) yielding variability among sites locally and nationwide.

Local agricultural practices such as timing of fertilizer applications likely influenced nitrate uptake dynamics, consistent with previous studies (Johnson et al. 2009; Sullivan et al. 2009). Longer uptake lengths, decreased uptake velocity and decreasing areal uptake were observed in summer corresponding to late summer applications as well as runoff of nitrogen rich fertilizers (Dinnes et al. 2002). Further, there was temporal variation in nitrate concentration (August > July > October). Increasing nitrate concentration has been linked to lower nitrate removal from

the water column to the benthos (Tank et al. 2006) and potential nitrate saturation (i.e., supply of nitrate exceeds the demand of the biota).

Overall, our findings complement the current understanding of pesticide uptake dynamics. Specifically, water quality programs, risk assessments, and remediation activities can determine a management plan according to the spatial and temporal variation of pesticides. Further, this study suggests that the octanol-water partition coefficient is not always a consistent predictor of pesticide movement in streams and other physicochemical characteristics (i.e., solubility) should be considered for prediction of pesticide movement in lotic ecosystems.

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CHAPTER III

EFFECTS OF ATRAZINE, METOLACHLOR, CARBARYL AND CHLOROTHALONIL ON BENTHIC MICROBES AND THEIR NUTRIENT DYNAMICS[1]

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Abstract

Atrazine, metolachlor, carbaryl, and chlorothalonil are detected in streams throughout the U.S. at concentrations that may have adverse effects on benthic microbes. Sediment samples were exposed to these pesticides to quantify responses of ammonium, nitrate, and phosphate uptake by the benthic microbial community. Control uptake rates of sediments had net remineralization of nitrate (-1.58 NO_3 μg gdm^{-1} h^{-1}), and net assimilation of phosphate (1.34 PO_4 μg gdm^{-1} h^{-1}) and ammonium (0.03 NH_4 μg gdm^{-1} h^{-1}). Metolachlor decreased ammonium and phosphate uptake. Chlorothalonil decreased nitrate remineralization and phosphate uptake. Nitrate, ammonium, and phosphate uptake rates are more pronounced in the presence of these pesticides due to microbial adaptations to toxicants. Our interpretation of pesticide availability based on their water/solid affinities supports no effects for atrazine and carbaryl, decreasing nitrate remineralization, and phosphate assimilation in response to chlorothalonil. Further, decreased ammonium and phosphate uptake in response to metolachlor is likely due to affinity. Because atrazine target autotrophs, and carbaryl synaptic activity, effects on benthic microbes were not hypothesized, consistent with results. Metolachlor and chlorothalonil (non-specific modes of action) had significant effects on sediment microbial nutrient dynamics. Thus, pesticides with a higher affinity to sediments and/or broad modes of action are likely to affect sediment microbes' nutrient dynamics than pesticides dissolved in water or specific modes of action. Predicted nutrient uptake rates were calculated at mean and peak concentrations of metolachlor and chlorothalonil in freshwaters using polynomial equations generated in this experiment. We concluded that in natural ecosystems, peak chlorothalonil and metolachlor concentrations could affect phosphate and ammonium by decreasing net assimilation, and nitrate uptake rates by decreasing remineralization, relative to mean concentrations of metolachlor and

chlorothalonil. Our regression equations can complement models of nitrogen and phosphorus availability in streams to predict potential changes in nutrient dynamics in response to pesticides in freshwaters.

Introduction

Agricultural activities, such as crop protection via pesticides, are increasing in response to global human population growth (projected to reach 9 billion by 2050; Enserink et al. 2013). In the last decade, U.S. pesticide sales have increased ~10 % with 80% of these pesticides used for agricultural activities (Enserink et al. 2013). The continued growth of the human population, coupled with the need for more efficient agricultural practices, will undoubtedly yield future increases in the occurrence of pesticides in freshwaters. Further, despite decades of research on agricultural pesticides, recent calls to action have highlighted the need to fill critical knowledge gaps in our understanding of how pesticides may adversely affect aquatic ecosystems (Kohler and Triebskorn, 2013; Murray et al. 2010).

Once in the aquatic ecosystem, pesticides may have adverse effects on organisms ranging from direct toxicity to indirect effects such as changes in growth or behavior (Relyea, 2006). At higher concentrations, such as those following spring runoff, direct mortality results across diverse organisms including tadpoles (Relyea, 2006), bluegill (Munn et al. 2001), and benthic organisms (e.g., amphipods and chironomids; Liess and Ohe, 2005). However, at lower concentrations, sub-lethal effects can result in altered respiration rates (McMahon et al. 2012), organismal growth (Relyea, 2006) and fecundity (Kashian and Dodson, 2002). In streams, benthic microbes are an important component of aquatic ecosystems and are integral in nutrient and energy dynamics (Covich et al. 1999). For example, nitrate, ammonium and phosphorus are cycled by benthic microbes through assimilation and remineralization (Dodds et al. 2002). These processes are influenced by N concentration in freshwaters (Bernot and Dodds, 2005; Earl et al. 2006). At saturated conditions, such as those in agricultural streams with high input of N from fertilizer runoff, more N is available, due to microbial uptake saturation (i.e., biota have reach

their N demand; Davis and Wayne, 1999) or an increase in heterotrophic mineralization (Bernot and Dodds, 2005). Further, at increasing N concentrations, PO_4 often becomes a secondary limiting nutrient (Earl et al. 2006).

In the Midwestern U.S., two herbicides (atrazine and metolachlor), one insecticide (carbaryl) and one fungicide (chlorothalonil) have both high usage rates and prevalence in receiving waters (USEPA, 1987; Kolpin et al. 1998; Larson et al. 1999; Gilliom et al. 2006). Atrazine is a triazine herbicide used predominantly in corn production for control of broadleaf and grassy weeds (Kruger et al. 1996) with a half-life in water at pH seven of 86 days (University of Hertfordshire, 2013). Metolachlor is a chloroacetanilide herbicide that inhibits mitosis and cell division (University of Hertfordshire, 2013). Metolachlor is stable in water at pH seven (University of Hertfordshire, 2013). Atrazine and metolachlor were detected in U.S. freshwaters at peak concentrations of 201µg/L and 77.6 µg/L (Table 9). Carbaryl is a carbamate family insecticide that inhibits the enzyme acetylcholinesterase (University of Hertfordshire, 2013), with a half-life in water of 12 days at pH seven (University of Hertfordshire, 2013). Chlorothalonil is a fungicide used in U.S. agriculture; it is stable in water at pH seven (University of Hertfordshire, 2013). Its mode of action is by binding to glutathione and negatively affecting cellular respiration (McMahon et al. 2012). Carbaryl and chlorothalonil were detected in U.S. freshwaters at peak concentrations of 4.8 µg/L and 0.3 µg/L (Table 9).

These pesticides are detected in freshwater ecosystems at concentrations that adversely affect biota and human health (Table 10). However, research has focused primarily on the impacts of agricultural pesticides on fish and invertebrates; little is known about how exposure to pesticides may directly influence benthic nutrient dynamics and overall ecosystem function (Beketov et al. 2013). For example, ecotoxicology studies addressing the effects of pesticides focus primarily on

non-benthic vertebrates (e.g., bluegill; Munn et al. 2001), and benthic invertebrates (e.g., amphipods and chironomids; Liess and Ohe, 2005) with few studies conducted on sediment microbial dynamics (Satsuma, 2006). Benthic microbial communities influence nutrient cycling (e.g., uptake, remineralization) by affecting fluxes as consumers or sources. Thus, benthic microbial communities are an important component of the freshwater ecosystem (Kemp and Dodds, 2002). These nutrient dynamics are affected by the presence of pesticides (Gramlich and Davis, 1967). Specifically, pesticides can reduce microbial activity that contributes to nutrient cycling (e.g., *Volvox* spp., *Botryococcus* spp., *Synedra* spp.; Neumann and Dudgeon, 2002), and change species composition by favoring microbes with enhanced pesticide degradation capacities. Also, pesticides can become nutrient sources by providing carbon, nitrogen or phosphorus to some microorganisms (Tappin et al. 2012), and alter nitrogen and/or phosphorus cycles (Doddamani and Ninnekar, 2001). Thus, there is a need to understand the direct effect of pesticides on sediment nutrient dynamics, and how these changes can affect whole-ecosystem pools and fluxes of nutrients (Brown et al. 2004).

We measured the effects of atrazine, metolachlor, carbaryl and chlorothalonil on benthic microbial nutrient dynamics by quantifying net assimilation and remineralization rates of ammonium, nitrate and phosphate in laboratory mesocosms. We hypothesized that pesticides with a broad mode of action and higher affinity to organic matter such as chlorothalonil (disruption of cellular respiration, log K_{ow}: 2.9) and metolachlor (inhibitor of mitosis and cellular division, log K_{ow}: 3.4) would decrease microbial nitrate and phosphate uptake rates. In contrast, pesticides with more specific modes of action and higher affinity to the aqueous phase (atrazine: blocks photosynthesis, log K_{ow}: 2.7 and carbaryl: inhibitor of synaptic activity, log K_{ow}: 2.4) were predicted to have no effect on nutrient cycling. Further, natural resources managers and

stakeholders would be able to make general predictions of agricultural pesticides effects on the microbial community based on the mode of action and water/solid affinities of these pesticides.

Methods

Experimental mesocosms

Sediment and water collection was conducted in May 2012 at Ball State University field station property of Jakes Creek - Cooper farm/Skinner field (40.234493, -85.45235) and approval for these experiments was received following the appropriate procedures. This sample collection did not involve endangered or protected species. Jakes Creek is a 3rd order agriculturally-influenced stream with adjacent row crops (i.e., corn and soybean) in Muncie, Indiana within the Upper White River Watershed (UWRW). During the sampling time, Jakes Creek water temperature was 17°C, pH 7.92, depth 5 cm (at sampling location), and discharge was 37 L/s. - While stream samples were not collected for pesticides analysis at the time of this experiment; stream water and sediment samples were collected one week prior to this study at the same site that shows atrazine and metolachlor concentrations were below detection limits. Filtered water samples (ten 1000 mL and one 200 mL) were collected from the stream thalweg using a 60 mm syringe and subsequently filtered (Whatman© glass fiber filter; 0.7 μm nominal pore size) into acid-washed Nalgene© bottles. The 200 mL Nalgene plastic bottle was used to determine initial concentrations of nitrate, ammonium, and phosphate. A composite sediment sample (~2000 cm^3) was randomly collected from the top 5 cm of the stream benthos and placed into three Nalgene plastic bottles. Sediment samples were transported on ice and subsequently combined and homogenized using a USGS no. 5 sieve in the laboratory. Homogenized sediment

(20 cm^3) and 60 ml filtered stream water were placed into each of 160 laboratory mesocosms (Fisherbrand sterile urine cup, 120 ml).

Stock solutions were prepared for atrazine (Atrazine 4L, 42.2% purity, Loveland, CO, US), metolachlor (Me-too-lachlor II, 84.4% purity, Drexel Chemical Company, TN, US), carbaryl (Sevin XLR Plus, 44.1% purity, Bayer, NC, US), and chlorothalonil (Bravo, 54% purity, Syngenta, NC, US) to achieve final stock concentrations of 10,000 µg/L for atrazine and metolachlor, 5,000 µg/L for carbaryl, and 8,000 µg/L for chlorothalonil. Aliquots from each stock solution were added to mesocosms to reach ten target treatment concentrations for each pesticide with four replicates for each treatment.

Mean and peak atrazine (2 µg/L, 201 µg/L) and metolachlor (1 µg/L, 78 µg/L) are detected at concentrations ~ 2 - 3 orders of magnitude higher than carbaryl (0.01 µg/L, 5 µg/L) or chlorothalonil (0.07 µg/L, 0.3 µg/L) throughout the U.S. (14-17). Thus, the treatment concentrations used were selected to include these environmentally relevant concentrations and appropriately represent pesticide occurrence in streams. Treatment solutions used in this study ranged from 0 µg/L (control) to the maximum concentrations detected in U.S. freshwaters for atrazine (200 µg/L), metolachlor (80 µg/L), carbaryl (4 µg/L) and chlorothalonil (0.5 µg/L) (Table 10).

Water from each mesocosm was removed after 24 h using a 10 mL syringe, subsequently filtered as above and placed into vials (two analytical replicates ~5 ml) for analysis of nitrate and phosphate via ion chromatograph (DIONEX, ICS-3000). The colorimetric phenol-hypochlorite technique (APHA, 1995; Aminot et al. 1997) was used to quantify ammonium concentrations. Initial concentrations (background) of nitrate, ammonium, and phosphate were also analyzed

following the analytical methods above. Sediment dry mass in each mesocosm was quantified using an analytical balance (OHAUS, Adventurer SL AS64).

Data analysis

Nutrient uptake rates were calculated for phosphate, ammonium and nitrate as changes in concentration over time (24 h) per g of dry mass (sediment) in response to treatments as (22):

$$\textbf{\textit{Nutrient uptake rate}} = \frac{(Cf - Ci) \cdot V}{T \cdot gdm}$$

Where: Cf = Final concentration (mg/L); *Ci* = Initial concentration (mg/L); *V* = Volume (L) in the jar*T* = time (h); *gdm* = g dry mass (g). Negative nutrient uptake rates indicated net remineralization of nutrients and positive nutrient uptake rates indicated net assimilation of nutrients (Table 11). Uptake rates were divided by the average of the control treatment for each pesticide (N = 16) to assess the effects of different treatments for a particular pesticides. Thus, in this study, a response ratio relative to controls was used as the response variable. Further, data were log-transformed to meet normality assumptions for statistical analyses. SigmaPlot© 12.0 software was used for linear and nonlinear regression analyses of response to pesticide concentration. The Akaike Information criterion (AIC) was used to select the best fit model among the different polynomial candidate models. Further, to develop predictive models of microbial response to pesticides in agricultural waters mean and peak concentrations of metolachlor and chlorothalonil throughout U.S. freshwaters (Table 9) were used to calculate nitrogen and phosphorus uptake rates.

Results

Nitrate, phosphate, and ammonium uptake rates varied less than one order of magnitude across pesticide treatments (Table 11). Phosphate uptake rates were three orders of magnitude greater than ammonium uptake rates across treatments, though both phosphate (mean = 180.56 $\mu g\ gdm^{-1}\ h^{-1}$) and ammonium (mean = 0.34 $\mu g\ gdm^{-1}\ h^{-1}$) uptake rates were net assimilative (Table 11). In contrast, we observed net remineralization of nitrate (mean = -32.22 $\mu g\ gdm^{-1}\ h^{-1}$) across pesticides (Table 11). Overall, these nutrient dynamics are expected in nitrogen-saturated agricultural ecosystems (i.e., microbial nitrate assimilation is saturated and PO_4 becomes a limiting nutrient).

Nitrate dynamics

Control nitrate uptake rate was ~20x higher on average than nitrate uptake influenced by pesticides (i.e., increasing remineralization in presence of pesticides). Nitrate uptake rates in response to atrazine, metolachlor, and carbaryl treatments exposure ranged from net assimilation (consumption/removal) to remineralization (source/addition) in the water column of our mesocosms (Table 11). In contrast, increasing concentrations of chlorothalonil yielded increasing nitrate remineralization ($p = 0.02$, $r^2 = 0.83$, Figure 15A). No other pesticides had significant effects on nitrate uptake rates ($p > 0.05$).

Phosphate dynamics

Phosphate uptake rates in response to pesticides exposure was ~100x higher than control phosphate uptake rates. Thus, phosphate assimilation increased in the presence of atrazine, metolachlor, carbaryl, and chlorothalonil relative to the control (Table 11). Further, phosphate

uptake was negatively related to increasing chlorothalonil concentration ($p < 0.001$, $r^2 = 0.87$, Figure 15B). Similarly, the metolachlor effect on phosphate uptake rate ($p = 0.005$, $r^2 = 0.91$, Figure 15C) followed a cubic relationship with increasing phosphate uptake at lower concentrations (0 to 10 µg/L), and decreasing rates at higher concentrations (10 to 80 µg/L). No other pesticides resulted in significant effects on phosphate uptake rates ($p > 0.05$).

Ammonium dynamics

Ammonium uptake rates in response to pesticides exposure was ~10x higher than control ammonium uptake rate. Thus ammonium assimilation increased in the presence of atrazine, metolachlor, carbaryl, and chlorothalonil relative to the control (Table 11). The ammonium uptake rate varied in response to metolachlor treatments ($p = 0.023$, $r^2 = 0.83$, Figure 15D), increasing with lower concentrations of metolachlor (0 to 10 µg/L), followed by a decline at higher concentrations (10 to 80 µg/L). No other pesticides had significant effects on ammonium uptake rates ($p > 0.05$).

Predicting nutrient response to pesticides

Changes in stream ecosystem nitrogen and phosphorus uptake rates were modeled across metolachlor and chlorothalonil concentrations detected throughout the U.S. (Figure 15). These changes were generated with polynomial regressions from this experiment ($p < 0.05$). The predicted ammonium uptake (0.52 NH_4 µg gdm^{-1} h^{-1}) in response to mean metolachlor concentrations was ~20 % higher than ammonium uptake (0.42 NH_4 µg gdm^{-1} h^{-1}) at peak metolachlor concentrations. At mean concentrations of metolachlor, the predicted phosphate uptake (212.2 PO_4 µg gdm^{-1} h^{-1}) was ~40 % lower than phosphate uptake (372.41 PO_4 µg gdm^{-1} h^{-1}) at peak metolachlor concentrations. At mean and peak chlorothalonil concentrations, there is

net remineralization of nitrate (-71.99 NO_3 µg gdm^{-1} h^{-1} and -41.87 NO_3 µg gdm^{-1} h^{-1}, respectively). Further, there was net assimilation of phosphate in response to mean and peak concentrations of chlorothalonil (207.88 and 77.81 PO_4 µg gdm^{-1} h^{-1}, respectively).

Overall, there was decreased ammonium assimilation and remineralization of nitrate in the presence of peak concentrations of metolachlor and chlorothalonil (Figure 15). Phosphate assimilation increased at peak concentrations of metolachlor and decreased at peak concentrations of chlorothalonil (Figure 15). Peak chlorothalonil is predicted to have the greatest effect on phosphate and nitrate uptake rates, by decreasing net assimilation and remineralization, respectively, over 50% relative to mean concentrations of these pesticides.

Discussion

Research focuses primarily on the impacts of agricultural pesticides on fish and invertebrates (Munn et al. 2001; Liess and Ohe, 2005); however, little is known about effects on microbial communities (Beketov et al. 2013). Our ecotoxicological research showed that benthic microbes' nutrient uptake rate response is likely a function of pesticide chemical characteristics, and how these changes can affect nutrient dynamics, due to nutrient availability. Variation in nutrient uptake rates is likely a result of differences in baseline nutrient concentrations and the biotic community. For example, control ammonium uptake rates in this study (0.026 µg NH_4 gdm^{-1} h^{-1}) were ~3x lower than control ammonium uptake rates in Bunch and Bernot (Bunch and Bernot, 2012). These results suggest dissimilar nutrient demand due to unique biotic communities and nutrient conditions across sampling sites and temporal variability.

In our study, mean ammonium uptake rate was ~1.5x higher than ammonium uptake under enriched nitrate conditions and ~2.5x lower under enriched ammonium conditions reported in

Bunch and Bernot (Bunch and Bernot, 2012). Thus, ammonium uptake is higher in the presence of pesticides than under enriched nitrate conditions and lower under enriched ammonium conditions, due to microbial adaptations to nutrient availability and metabolic responses in presence of pesticides. Our nitrate uptake results were similar to yields with net remineralization under enriched ammonium conditions reported in Bunch and Bernot (Bunch and Bernot, 2012), though remineralization was 10 times lower than rates measured in response to pesticides in our study.

The effects of metolachlor and chlorothalonil on nutrient uptake rates suggest a unique biotic community at this site, represented by mostly heterotrophic benthic microbes. Our results suggest a toxic effect of metolachlor and chlorothalonil on the benthic microbial community that is reflected by the increased nitrate remineralization, and reduced assimilation of ammonium and phosphate. This increase in remineralization rates could be an outcome of cellular lysis or a stress mechanism (Bronk and Ward, 1999). Further, a biotic community characterized by autotrophs (Kemp and Dodds, 2002) had ammonium and nitrate uptake rates three orders of magnitude higher than benthic uptake in our study; possibly, under these study conditions, primary producers have a higher assimilation rate than heterotrophic benthic microbes in response to the available forms of nitrogen (Kemp and Dodds, 2002). Further, control phosphate uptake rates in our study were ~5x lower than rates previously measured with phosphorus enrichment (0.1 -2 mg/L; 34). Thus, phosphate is likely a limiting nutrient in our system. However, in presence of pesticides, phosphate uptake was two orders of magnitude higher than the rates measured under phosphorus limiting conditions (Klotz, 1985) possibly, in addition to available phosphorus in the water column, microbes were potentially degrading pesticides as a source of phosphorus (Cook et al. 1978).

Nutrient dynamics in the presence of pesticides are dependent on the physicochemical characteristics (e.g., sorption kinetics, modes of action) of each pesticide. Sorption kinetics of pesticides and their corresponding index (Octanol-water partition coefficient, K_{ow}) determine the affinity of organic contaminants to either the water column or sediments (Wauchope et al. 2002). Atrazine and carbaryl have a higher affinity to the aqueous phase (Log K_{ow}: 2.7 and 2.4, respectively), relative to metolachlor and chlorothalonil (Log K_{ow}: 3.4 and 2.9, respectively). Thus, atrazine and carbaryl are likely more prevalent in the water column and less available to the sediment microbial communities, with minimal effect on benthic microbial activity. In contrast, metolachlor and chlorothalonil have a higher affinity to solids and higher prevalence in sediment, potentially affecting benthic microbial activity. Our interpretations of pesticide availability based on their water/solid affinities supports our results of no effects for atrazine and carbaryl (Log $K_{ow} < 2.7$, $p > 0.05$) within the tested range, decreasing nitrate remineralization and decreasing phosphate uptake in response to chlorothalonil, and decreasing ammonium and phosphate uptake in response to metolachlor (Log $K_{ow} > 2.9$, $p < 0.05$).

Nutrient dynamics are also affected by the pesticide mode of action. Atrazine and carbaryl have specific modes of action; atrazine blocks photosynthesis, and carbaryl inhibits the activity of acetylcholinesterase, an enzyme of insects, fish, mammals (Durieux et al. 2011). Thus, the specificity of atrazine and carbaryl, and a sediment microbial community dominated by heterotrophs with no synaptic activity (Pester et al. 2004) may explain the lack of significant effects of these pesticides on benthic microbes within the tested range (Table 11). In contrast, metolachlor and chlorothalonil are broad spectrum pesticides (University of Hertfordshire, 2013). In our study, ammonium and phosphate uptake rates decreased with increasing metolachlor concentrations. These decreasing ammonium and phosphate uptake rates could be

due to metolachlor inhibition of mitosis and cell division. Similarly, phosphate and nitrate uptake rates were affected by chlorothalonil; possibly this fungicide affects benthic microbes by disrupting cellular respiration. Thus, pesticides with non-specific modes of action (e.g., metolachlor, chlorothalonil) are more likely to have a significant effect on nutrient dynamics of sediment microbes, consistent with our study results.

Metolachlor and chlorothalonil not only affect nutrient dynamics of the sediment microbial community; they can also affect other processes. In our study, at increasing concentrations of chlorothalonil, there is a decrease in phosphate uptake, thus there is more phosphate available for organismal consumption. In agricultural streams, where phosphorus is the limiting nutrient (Correll, 1999), increasing availability of phosphate can lead to algal blooms, eutrophication, hypoxia, loss of biodiversity (e.g., fish kills), and loss of aesthetic value of these habitats (Conley et al. 2009). In contrast, in the presence of peak concentrations of metolachlor, there is an increase in phosphate assimilation, which could mitigate the excess phosphate in streams (Gachter and Meyer, 1993). Similarly, there is an increase of ammonium availability due to inhibited uptake rates in response to metolachlor, which in turn could potentially increase biological activity of pesticide resistant microbes (Cook et al. 1978; Doddamani and Ninnekar, 2001; Satsuma, 2006; Tappin et al. 2012). Nitrate remineralization also decreases in response to chlorothalonil, reducing nitrate availability, and potentially further mitigating excess nitrogen in these habitats (Zogg et al. 2000).

Mesocosms have a critical role in understanding the mechanisms driving ecological processes (Frost et al. 2001; Spivak et al. 2011) and provide a bridge between smaller, better controlled experiments and the larger freshwater ecosystems (Stewart et al. 2013). For example, (Spivak et al. 2011) revealed that the effects of nutrients on primary producers are similar in

artificial habitats across five orders of magnitude in size. Also, (Stewart et al. 2013) mentioned that mesocosms help disentangle direct from indirect effects over scale. Thus, our conclusions try to bridge what we observed in the laboratory level and what could potentially occur at an agricultural influenced stream level.

Pesticide occurrence and concentrations in streams are dictated primarily by land-use. Streams receiving runoff from agricultural landscapes frequently have the highest concentrations of pesticides, compared to forested, mixed-use and urban lands (Rinella and Janet, 1998). These high concentrations occur as pulses that are coupled by the seasonality of agricultural practices (Larson et al. 1999). Peak concentrations of metolachlor and chlorothalonil are detected from April to August (Larson et al. 1999). Thus, the effects of these pesticides on nutrient dynamics are highest during this critical time. At peak metolachlor and chlorothalonil concentrations there is a decrease of predicted ammonium assimilation and nitrate remineralization. Further, at peak concentrations of metolachlor there is increased phosphate assimilation and at peak concentrations of chlorothalonil there is decreased phosphate assimilation (Figure 15).

Our findings demonstrate individual effects of these pesticides on sediment nutrient dynamics that are likely driven by a pesticides' mode of action and water/sediment affinities. More studies are required to understand the net effect on ecosystems and to address their synergistic or antagonistic effects as mixtures. The regression equations we generated can complement models of nitrogen and phosphorus availability in streams to predict the potential changes in nutrient dynamics in response to increasing presence of pesticides in lotic ecosystems.

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CHAPTER IV

EFFECTS OF INDIVIDUAL AND COMBINED PESTICIDE EXPOSURE TO EGESTION AND MOVEMENT OF COMMON FRESHWATER SNAILS, *PHYSA ACUTA* AND *HELISOMA ANCEPS*

Abstract

Pesticides are detected in streams at concentrations that may have adverse effects on aquatic organisms. These agrochemicals typically occur in streams as combinations, yet research has focused on the effects of individual pesticides. We studied the effects of atrazine, metolachlor, carbaryl, and chlorothalonil on aquatic gastropods *Physa acuta* and *Helisoma anceps* egestion and movement. We observed an 8-fold reduction in *P. acuta* egestion rates when exposed to individual and combined pesticide treatments relative to controls. For *H. anceps*, individual and combined pesticide treatments had no significant effects on egestion, highlighting differential species response. *H. anceps* movement did decline when exposed to atrazine, carbaryl, and chlorothalonil individually, though responses varied with exposure time. When combined, atrazine + metolachlor and carbaryl + chlorothalonil reduced *H. anceps* movement relative to control. In addition to pesticide physicochemical characteristics, it is important to consider exposure durations to better understand the effects of pesticides on aquatic organisms. Further, future risk assessments should incorporate multiple organisms to better represent response diversity.

Introduction

Increased pesticide use as result of increasing crop protection practices and global human population growth (Population Reference Bureau, 2015) has resulted in pesticide concentrations in freshwater ecosystems that may have adverse effects on aquatic organisms. At elevated concentrations, such as following runoff events, mortality of diverse organisms including bullfrogs (Relyea, 2006), cladocerans (Munn et al. 2001), and caddisfly (Liess and Ohe, 2005) has been observed. Further, at lower concentrations, sub-lethal effects can result in changes of physiological processes including behavior and growth in amphibians (Relyea, 2006), lower fecundity in cladocerans (Kashian and Dodson, 2002), reduction of decomposition rates in aquatic communities (McMahon et al. 2012), and lower nutrient uptake of sediment microbes (Elias and Bernot, 2014). Though previous research has documented adverse effects of individual compounds on aquatic organisms, few studies have evaluated the effect of multiple-stressors.

Pesticides are rarely applied individually in agriculture; rather, pesticides are used in combination at specific times during crop production (Gilliom et al. 2006). In the U.S., atrazine (triazine), metolachlor (chloroacetanilide), carbaryl (carbamate), and chlorothalonil (chloronitrile) have high usage rates (Solomon et al. 1996) prevalence in streams (Larson et al. 1999), and co-occur more often as mixtures than as individual pesticides (Gilliom, 2007). Thus, pesticide abundance overlaps in space and time as a result of usage and application (Smiley et al. 2014). Pre-emergent herbicides (atrazine and metolachlor) are usually detected following spring applications (Thurman et al. 1991) and are commonly applied in Midwestern U.S. (Kolpin et al. 1998). Insecticides (carbaryl) and fungicides (chlorothalonil) are applied multiple times

throughout the year to control pest outbreaks, and are commonly sprayed on the crops concurrently (e.g., asparagus, snap beans, sweet corn; NASS – USDA, 2005).

Pesticide effects on aquatic organisms have been observed in response to both individual and combined pesticide exposure. Individually, atrazine (3 µg/L) increases susceptibility of amphibians to trematode infection (Kohler and Triebskorn, 2013). Metolachlor (40 µg/L to 52 µg/L) might interfere with crayfish chemosensory stimuli (Wolf and Moore, 2002; Cook and Moore, 2008) and reduced biomass and growth of *Lemna gibba* (University of Hertfordshire, 2015). Carbaryl (0.5 µg/L) increased refuge time of *Ambystoma barbouri* (Rohr et al. 2003); and chlorothalonil (0.01 µg/L to 0.5 µg/L) increased nitrate remineralization of benthic microbes (Elias and Bernot, 2014). As mixtures, atrazine (10 µg/L) and metolachlor (10 µg/L) increased time to initiate metamorphosis in *Rana pipiens* (Hayes et al. 2006), and frequency of amphibians with thymic plaques (Hayes et al. 2006). In contrast, to our knowledge, no studies have been conducted on aquatic organism response to carbaryl and chlorothalonil mixtures.

Aquatic gastropods are commonly used in ecotoxicological studies to address the effects of pesticide exposure (see *Pseudosuccinea columella*, Tate et al. 1997; *Stagnicola elodes*, Koprovnikar and Walker, 2011; *Physella* spp., Baxter et al. 2011; *Potamopyrgus antipodarum*, Hock and Poulin, 2012). However, few studies are conducted on *Physa acuta* or *Helisoma anceps*, particularly in regards to pesticides mixtures. *Physa acuta* and *H. anceps* (subclass: Pulmonata) are ubiquitous aquatic gastropods in North America and are common in freshwater habitats across a range of human influence (Dillon et al. 2002; Thorp and Covich, 2009). These snails mature quickly (McCarthy et al. 2000), lay eggs in masses (Dillon et al. 2006) and graze on biofilm (algae, bacteria, fungus) growing on substrates (Hawkins et al. 1987), as well as detritus (Brady and Turner, 2010); thus fulfilling an important role as primary consumers and

decomposers (Newman et al. 1996). *Physa acuta* and *H. anceps* are also prey for diverse vertebrate and invertebrate organisms including crayfish (*Orconectes juvenilis*; Dickey and McCarthy, 2007), and pumpkinseed sunfish (*Lepomis gibbosus*; Justice and Bernot, 2014). Further, *P. acuta* and *H. anceps* are important intermediate hosts of parasites, including *Halipegus eccentricus, H. occidualis, Echinostoma trivolvis, Megalodiscus temperatus,* and *Fasciola hepatica* (Sapp and Esch, 1994) and can cause disease in wildlife (Gustafson and Bolek 2015), livestock (Case, 1953), and humans (Graczyk and Fried, 1998). Thus, research on the effects of pesticides on *P. acuta* and *H. anceps* is essential, due to their key role in nutrient cycling, functional link between primary producers and secondary consumers, parasite and disease transmission, and as model organisms (Dillon et al. 2011) for reproductive (Wethington and Dillon, 1993; Jordaens et al. 2007) and ecotoxicological studies (Bernot et al. 2005; Relyea, 2006; Bakry et al. 2011; Maredza and Naik, 2013; Basopo et al. 2014).

In this study, we measured the effects of individual and combined pesticides at environmentally relevant concentrations applied throughout the U.S. We observed the effects of two herbicides (atrazine and metolachlor), one insecticide (carbaryl), and one fungicide (chlorothalonil) on aquatic gastropods that are likely exposed to these pesticides in natural ecosystems. We measured egestion rates and movement rates of *P. acuta* and *H. anceps* to examine possible mechanisms influencing these processes. We predicted similar effects of pesticide exposure on *P. acuta* and *H. anceps* egestion due to similar feeding behavior (grazers), available food (biofilms and detritus), and habitats. Further, we hypothesized that pesticides that target primary producers (atrazine: photosynthesis; metolachlor: gibberellins and mitosis) would have no effect on snail egestion or movement. In contrast, we predicted pesticides that target

invertebrates (carbaryl: acetylcholinesterase and chlorothalonil: reduction of fungal intracellular glutathione) would reduce snail egestion rate and movement.

Methods

Physa acuta was collected from the White River (Muncie, IN; 40.1805 N 85.432 W). This area is surrounded by urban and forest landscape (oaks, maples, white ash, elm, sycamore). *H. anceps* was purchased through a commercial vendor (Meijer, Inc.). These snails were maintained in synthetic spring water filled aquaria at 20°C +/- 3°C and fed boiled spinach *ad libitum* and supplemented with Topfin tropical flakes fish food. Snails were maintained under a 16:8 h light:dark photoperiod for the duration of the experiments. Experimental mesocosms consisted of glass jars (150 mL) filled with 120 mL of synthetic spring water. One snail was placed into each jar at experiment start. Treatments were randomly assigned with four replicates each across seven treatments (n = 28) including: control, atrazine (200 µg/L), metolachlor (100 µg/L), carbaryl (100 µg/L), chlorothalonil (100 µg/L), atrazine + metolachlor (200 µg/L + 100 µg/L), and carbaryl + chlorothalonil (100 µg/L + 100 µg/L). Stock solutions were prepared for atrazine (Atrazine 4L, 42.2% purity, Loveland, CO, US), metolachlor (Me-too-lachlor II, 84.4% purity, Drexel Chemical Company, TN, US), carbaryl (Sevin XLR Plus, 44.1% purity, Bayer, NC, US), and chlorothalonil (Bravo, 54% purity, Syngenta, NC, US) to achieve final stock concentrations of 10,000 µg/L for atrazine and metolachlor, 5,000 µg/L for carbaryl, and 8,000 µg/L for chlorothalonil. Aliquots from each stock solution were added to mesocosms to reach target nominal treatment concentrations. Pesticide treatment concentrations were selected to represent peak concentrations detected throughout the U.S. (Table 12). Water changes for cultured snails as well as treatments concentration renewal were conducted twice weekly.

Pesticide effects on P. acuta and H. anceps egestion rates

Effects of pesticides on *P. acuta* and *H. anceps* egestion rates were estimated by weighing feces. Snails were starved for 24 h before the start of the egestion experiment to fully empty their intestines of fecal matter (*sensu* Bernot et al. 2005). Snails were then placed in individual mesocosms with freshwater and treatment solutions as well as 0.05 g of boiled spinach (wet mass) as a food source. After 24 h, fecal matter was removed with micropipettes, filtered onto filter paper, dried (60°C for 24 h) using a Model 30 GC laboratory oven, and weighed to the nearest milligram (Autobalance AD6, Perkin Elmer). Snails were blotted dry using Kimwipes (Kimberly-Clark) and weighed (Mettler AE260, Delta Range). Egestion rates (mg/g/h) were calculated as the amount of feces produced (mg) divided by the mass of each snail (g) per hour (h).

Pesticide effects on H. anceps movement rates

Snail movement was quantified for *H. anceps* in response to pesticide treatments (n = 7) after 24 h, 1 week and 2 weeks exposure to atrazine, metolachlor, carbaryl, and chlorothalonil (*sensu* Bernot et al. 2005). At each time point, snails were removed from mesocosms for movement measurement, and subsequently returned to their corresponding mesocosm. For each movement measurement, individual snails were placed in glass aquaria (50.8 x 27.9 x 30.5 cm) with 1 cm^2 square grid paper beneath (Brown et al. 2012). Synthetic spring water was added to the glass aquaria to a height of 5 cm. The snail was then placed with the aperture down on the bottom of the aquarium in the center of the grid paper. After 10 s of acclimation, grid lines that the snail crossed within 2 minutes were counted (grid lines crossed during the first 10 s were not

recorded). A total of four replicate snails from each treatment (n = 28) were assessed for movement at each time point (n = 3).

Data analysis

Physa acuta and *H. anceps* egestion rates

The egestion experiment was set up as a factorial design consisting of two levels of species (*P. acuta* and *H. anceps*) and seven levels of pesticide treatments (control, atrazine, metolachlor, carbaryl, chlorothalonil, atrazine + metolachlor, and carbaryl + chlorothalonil) with four replicates for each treatment (n = 28). Each individual snail species was matched with a corresponding pesticide treatment. Two-way analysis of variance was used to analyze the effects of pesticide treatments on snail species (*P. acuta* and *H. anceps*) egestion rates. The carbaryl + chlorothalonil treatment had 100% mortality in *P. acuta*; thus, it was not included in data analyses. Differences among treatments were assessed with Tukey multiple comparison tests. Analyses were conducted using SigmaPlot© 12.0 software.

Helisoma anceps movement rate

The movement experiment was set up as a factorial design with seven levels of pesticide treatments (control, atrazine, metolachlor, carbaryl, chlorothalonil, atrazine + metolachlor, and carbaryl + chlorothalonil) and three levels of pesticide exposure period (one day, one week, and two weeks) with four replicates for each treatment (n = 28). Each individual snail was matched with its corresponding pesticide treatment. Two-way repeated measures analysis of variance was used to analyze the effects of pesticide exposure period and pesticide treatments on *H. anceps* movement rate. Differences among treatments were assessed with Tukey multiple comparison tests. Analyses were conducted using SigmaPlot© 12.0 software.

Results

Physa acuta and *H. anceps* egestion rates

Snails egestion rate was different among species ($F_{1,42} = 79.15$, $p < 0.001$) and pesticide treatments ($F_{6,42} = 21.58$, $p < 0.001$). Further, there was a significant interaction between the effects of species and pesticide treatments ($F_{6,42} = 14.55$, $p < 0.001$). *Physa acuta* egestion rates were higher than *H. anceps* across treatments (Figure 16); and ranged from 0.04 - 0.5 mg feces/g snail/h (*P. acuta*) and 0.01 - 0.07 mg feces/g snail/h (*H. anceps*). Control egestion rate for *P. acuta* was 7x higher than control egestion rate for *H. anceps* ($F_{1,42} = 79.15$, $p < 0.001$). A similar trend was observed for *P. acuta* and *H. anceps* in response to pesticide exposure; where *P. acuta* egestion rates was 8x higher than *H. anceps* with metolachlor ($F_{1,42} = 79.15$, $p = 0.016$), 9x higher with carbaryl ($F_{1,42} = 79.15$, $p < 0.001$), and 7x higher with chlorothalonil ($F_{1,42} = 79.15$, $p < 0.001$). There were no significant differences between egestion rates of *P. acuta* and *H. anceps* when exposed to atrazine, and atrazine + metolachlor ($p > 0.05$). *Physa acuta* egestion was differentially influenced by herbicide (atrazine and metolachlor), insecticide (carbaryl), or fungicide (chlorothalonil) exposure. For the individual pesticides, *P. acuta* egestion rates were 7x lower with atrazine, 4x lower with metolachlor, 2x lower with carbaryl, and 2x lower with chlorothalonil relative to control ($F_{6,42} = 21.58$, $p < 0.001$; Figure 16). For the pesticide mixtures, atrazine + metolachlor exposure resulted in *P. acuta* egestion rates 12x lower than control ($F_{6,42} = 21.58$, $p < 0.001$). For *H. anceps*, individual and mixtures of atrazine, metolachlor, carbaryl, and chlorothalonil had no significant effects on egestion rates ($p > 0.5$). The significant interaction of species and pesticide treatments indicates that pesticide treatment effects on snail egestion rate are influenced by the snail species. Thus, we observed a no effect of pesticide exposure to *H. anceps* egestion and significant effect of pesticide exposure for *P. acuta* egestion rate.

Helisoma anceps movement rate

Overall, *H. anceps* movement rate was different among pesticide treatments ($F_{6,42} = 6.88$, $p < 0.001$) and pesticide exposure period ($F_{2,42} = 17.32$, $p < 0.001$). Further, there was not a significant interaction between the effects of pesticide treatments and pesticide exposure period ($p > 0.5$). Snail movement rate decreased when exposed to pesticide treatments (Figure 17A, Figure 17B). After pesticide exposure, snail movement rate ranged from 0 to 0.75 cm/min. For individual pesticides (Figure 17A), *H. anceps* movement was 2x lower with atrazine ($p = 0.005$), metolachlor ($p = 0.026$), and chlorothalonil ($p = 0.005$) exposure and 4x lower with carbaryl ($p < 0.001$) exposure relative to control. Similar trends were observed in snail movement in response to pesticide mixtures (Figure 17B). *H. anceps* movement was 3x lower with atrazine + metolachlor ($p = 0.005$), and 7x lower with carbaryl + chlorothalonil ($p <0.001$) relative to control. Pesticide exposure period affected snail movement rate differently. *H. anceps* movement rate decreased over time (n = 14 days) for both control and pesticide treatments with a more pronounced effect after one week and two weeks. Snail movement rate ranged from 0.13 to 0.75 cm/min after 24 h, from 0.13 to 0.56 cm/min after one week, and from 0 to 0.44 cm/min after two weeks. Snail movement rate was 2x lower after one week ($F_{2,42} = 17.32$, $p < 0.001$) and 4x lower after two weeks ($F_{2,42} = 17.32$, $p = 0.002$) relative to 24 h pesticide exposure.

Discussion

In our study, we measured the individual and combined effects of atrazine, metolachlor, carbaryl, and chlorothalonil on *P. acuta* and *H. anceps.* Both snail species egestion and movement were negatively affected by individual and combined atrazine, metolachlor, carbaryl,

and chlorothalonil exposure at environmentally relevant concentrations. Control egestion rate for *P. acuta* was greater than *H. anceps*; likely influenced by species innate assimilation efficiency (Studier et al. 1975; Barnese et al. 1990), and species sensitivity (Hylleberg, 1975; Bernot et al. 2005; Suski et al. 2012). Traditionally, species sensitivity to toxicants has been predicted through a taxonomic-based approach (Rubach et al. 2009), where related species are expected to have a similar response to toxicant exposure. However, species single traits as well as combinations of traits are better predictors of the effects to toxicant exposure (Rubach et al. 2009). For example, thiacloprid exposure (3.3 µg/L) to macroinvertebrates with low sensitivity combination of traits resulted in short term harmful effects (i.e., reduction of abundance and richness). In contrast, species with high sensitivity combination of traits exposed to thiacloprid concentrations of 0.1 µg/L resulted in permanent adverse effects (Liess and Beketov, 2011). Although individual or combined traits of *P. acuta* and *H. anceps* sensitivity to atrazine, metolachlor, carbaryl, and chlorothalonil have not been identified, we observed higher egestion rates of *P. acuta* than *H. anceps* when exposed to pesticide treatments, as well as no significant effect of pesticide exposure on *H. anceps* egestion. Thus, consistent with a species trait approach, our results suggest dissimilar species sensitivity to environmental stressors (Hylleberg, 1975; Bernot et al. 2005; Suski et al. 2012).

Contrary to hypotheses, we observed a decrease in *P. acuta* egestion rates when exposed to any pesticide. This may be partially explained by narcosis (Wezel and Opperhuizen, 1995; Ren, 2002; Roberts and Costello, 2003) which is a non-specific mode of action where a chemical does not interact with a particular receptor in an organism (Verhaar et al, 1992; Cleuvers, 2002). While no adverse outcome pathway (Ankley et al. 2010; Vinken, 2013) has been developed for these pesticides on freshwater snails; we presume that the narcotic effect of pesticides on *P.*

acuta egestion is likely a result of disruption of Van der Waals interactions between lipid and protein components within the membrane (Frank and Lieb, 1990; Yamakura et al. 2001). Damage of cell membrane could increase cell susceptibility to lysis due to abnormal ion fluxes and dysfunction of organelles (Kinter and Pritchard, 2011); which could lead to cardiac or hepatic failure, thus impairing snail egestion. However, we observed no effect of individual or combined pesticide treatments in *H. anceps* egestion rates. Similarly, no significant effects on growth and fecundity were observed for *Physella* sp. exposed to atrazine concentrations of 0, 1, 10, 30 and 100 µg/L (Baxter et al. 2011), or *Helisoma trivolvis* exposed to 0.51 mg/L of carbaryl (Relyea, 2005). Thus, we presume that pesticide exposure concentrations, as well as species sensitivity played an important role in differential species response.

Pesticide effects on organism are likely influenced by compound mode of action (Elias and Bernot 2014), species sensitivity to toxicants (Van Straalen and Denneman, 1989; DeLorenzo et al. 2009), and pesticide exposure period (Ashauer et al. 2009; Maltby et al. 2009). We predicted pesticide mode of action and pesticide exposure period as the main factors influencing invertebrate response. However, *H. anceps* movement was not influenced by these factors. Similar to the egestion experiment, snail movement rate decreased when exposed to any pesticide (i.e., narcosis). Also, we observed a decrease of snail movement rate through time (14 days) likely due to decline in *H. anceps* fitness. Snail fitness can be assess by quantifying fitness traits such as fecundity (Coutellec and Lagadic, 2006), survival (Wethington and Dillon, 1997), and movement (Bernot et al. 2005). Further, snail fitness decreases in response to environmentally related stress (Coutellec and Lagadic, 2006) including physicochemical parameters (Hunter, 1990), predation (Dewitt, 1998), contaminants (Justice and Bernot, 2014), and food availability (Auld and Henkel, 2014). Our study provided steady room temperature

(~20 ºC), did not include predation stimulus or limited food. Further, contaminant (i.e., pesticides) did not have a significant interaction effect with exposure period. However, optimal fitness (e.g., growth, egg production) of *Helisoma duryi* occurs between 26 ºC to 28ºC (El-Emam and Madsen, 1982). Thus, the decline of snail movement through time is likely influenced by the lower temperatures of our mesocosms.

In our study, we cannot use "concentration addition" (mixture of pesticides with the same mode of action; Deneer, 2000) or "independent action" (mixture of pesticides with dissimilar mode of action; Cedergreen et al. 2008) to classify the effects of combined atrazine and metolachlor or combined carbaryl and chlorothalonil. However, atrazine + metolachlor had a greater effect on *P. acuta* egestion than individual atrazine (blocks photosynthesis) and metolachlor (inhibits protein and gibberellins production). A comparable trend was observed on *Rana pipiens* when exposed to individual and combined atrazine and S-metolachlor. *R. pipiens* tadpoles exposed to atrazine and S-metolachlor simultaneously had a greater reduction of larval development and growth than individual atrazine or S-metolachlor (Hayes et al. 2006). Thus, our results suggest that changes in *P. acuta* egestion rates are greater when exposed to pesticide mixtures than individual pesticides, although the specific mechanisms cannot be elucidated from our current research.

Atrazine, metolachlor, carbaryl, and chlorothalonil not only affect *P. acuta* egestion and *H. anceps* movement; they can also indirectly affect food webs and prey-predator interactions. Lower egestion rates for *P. acuta* when exposed to pesticides may reduce nitrogen and carbon availability, thus affecting nutrient fluxes and algal biomass (Conley et al. 2009). Further, decreasing movement of *H. anceps* may alter snail antipredator behavior including hiding or avoidance from predatory fish, amphibians, and insects (Covich et al. 1994; Justice and Bernot

2014), and grazing behavior (Turner and Montgomery, 2003; Bernot et al. 2005). Thus, in addition to pesticide mode of action, it is important to consider species sensitivity to pesticides coupled with different exposure periods to better understand the potential adverse effects of atrazine, metolachlor, carbaryl, and chlorothalonil to ecosystems. Similarly, future risk assessments and ecotoxicological studies should include more than one species of snails to have a better representation of the freshwater gastropods community given their importance in nutrient cycling, foods webs, and parasite transmission.

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CONCLUSIONS

The objective of this research was to determine: 1) What are the factors that affect atrazine, metolachlor, carbaryl, and chlorothalonil abundance and uptake metrics in streams?; and, 2) What are the effects of atrazine, metolachlor, carbaryl, and chlorothalonil on aquatic organisms. We addressed these questions through four chapters. In Chapter I, we assessed the factors that influence atrazine and metolachlor abundance at the regional scale (descriptive sampling) and national scale (meta-analysis). For both regional and national assessments, peak concentrations of atrazine and metolachlor occurred in spring (April to June) matching spraying schedules. Spatial variation of atrazine and metolachlor concentrations was likely influenced by state crop and pesticide selection. Further, atrazine and metolachlor concentrations were influenced by different stream physicochemical parameters depending on the spatial scale. At the regional scale, dissolved metolachlor was negatively related with phosphate and oxygen concentrations, and sediment-bound metolachlor was positively correlated with dissolved organic carbon concentrations. At the national scale, atrazine was positively related to temperature and negatively related to pH, while metolachlor was positively related to ammonium concentrations. Thus, it is necessary to consider local stream parameters to fine tune predictive models developed at the national scale.

In Chapter II, we assessed the factors that influence atrazine, metolachlor, and carbaryl transport in streams through enrichment experiments as well as nitrate for comparison. Overall, uptake length (S_w) varied less than one order of magnitude across pesticides with the highest transport (S_w) for atrazine suggesting greater transport to downstream ecosystems. Increasing nitrate transport, with corresponding decreasing demand of nitrate, suggests that the supply of nitrate exceeds the demand of aquatic organisms in our study site, Jakes Creek. Similarly,

pesticide uptake metrics were dictated by pesticide concentrations and better predicted by compound solubility.

In Chapter III, we quantified nutrient (NO_3, PO_4, NH_4) uptake of benthic microbes when exposed to atrazine, metolachlor, carbaryl, and chlorothalonil. Our interpretation of pesticide availability based on their water/sediment affinities supports our results of no effects for atrazine and carbaryl, decreasing nitrate remineralization and phosphate assimilation in response to chlorothalonil, and decreased ammonium and phosphate uptake in response to metolachlor. Further, atrazine targets autotrophs, and carbaryl targets synaptic activity; thus, we hypothesized no effects on benthic microbes, consistent with results. Metolachlor and chlorothalonil (non-specific modes of action) had significant effects on nutrient dynamics of benthic microbes. Thus, pesticides with a higher affinity to sediments and/or broad modes of action were more likely to affect nutrient dynamics of benthic microbes than pesticides more soluble in water or having specific modes of action.

In Chapter IV, we assessed the effects of individual and combined atrazine, metolachlor, carbaryl, and chlorothalonil on *Physa acuta* and *Helisoma anceps* egestion rates and movement. Overall, pesticide exposure to these snails highlighted differential species response. *Physa acuta* egestion rate was reduced 8x by pesticide exposure (individual and combined), and though no effect was observed for *H. anceps*. Further, *H. anceps* movement decline when exposed to atrazine, carbaryl, and chlorothalonil individually, though responses varied with exposure time. Thus, in addition to pesticide physicochemical characteristics, it is important to consider exposure durations to better understand the effects of pesticides on aquatic organisms. Further, future risk assessments should incorporate multiple organisms to better represent response diversity.

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FIGURES

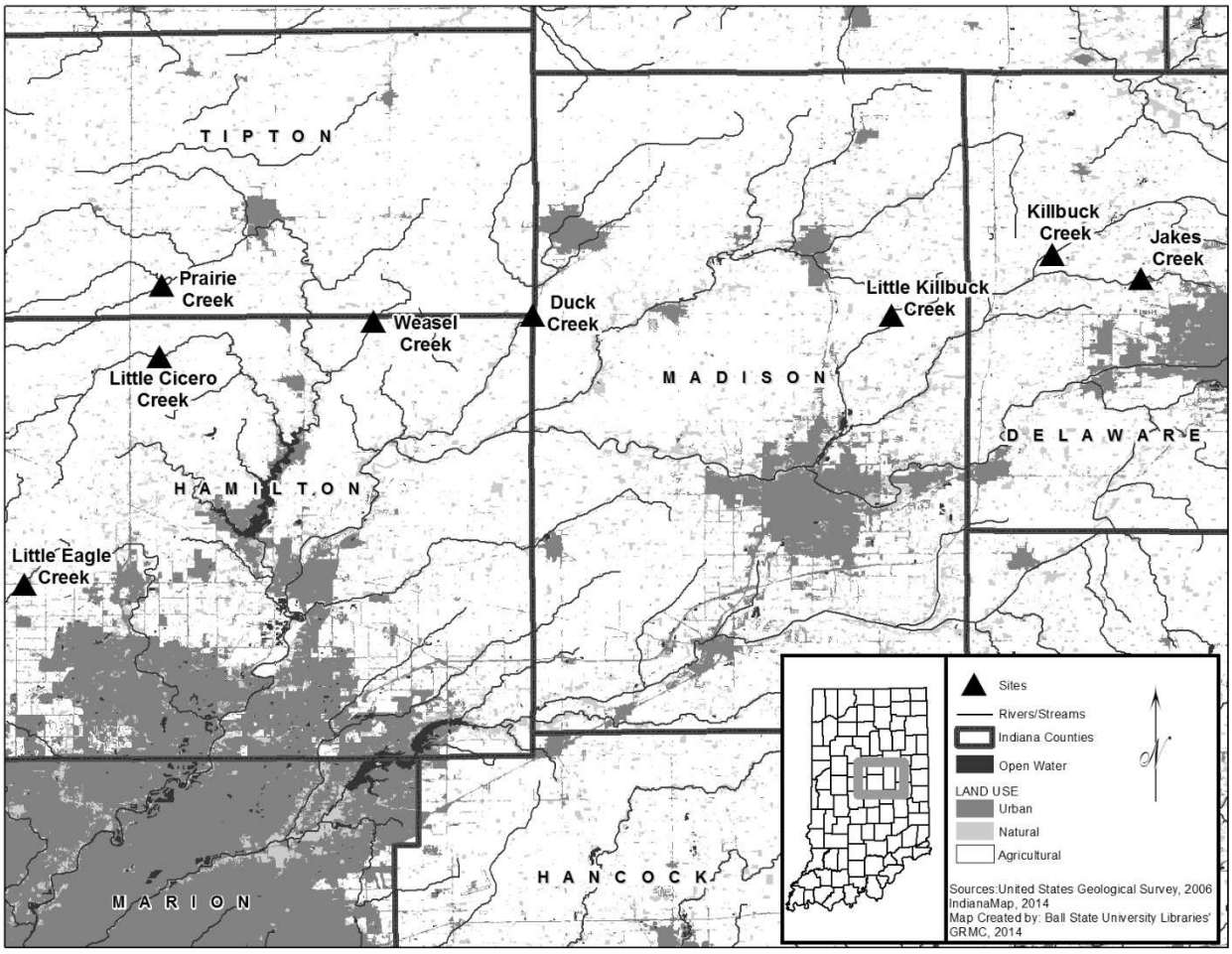

Figure 1: Regional study sites in the Upper White River Watershed (UWRW) of central Indiana with the surrounding land use denoted as urban, natural, and agricultural. Samples were collected monthly at each site from October 2011 to September 2012 (N = 12).

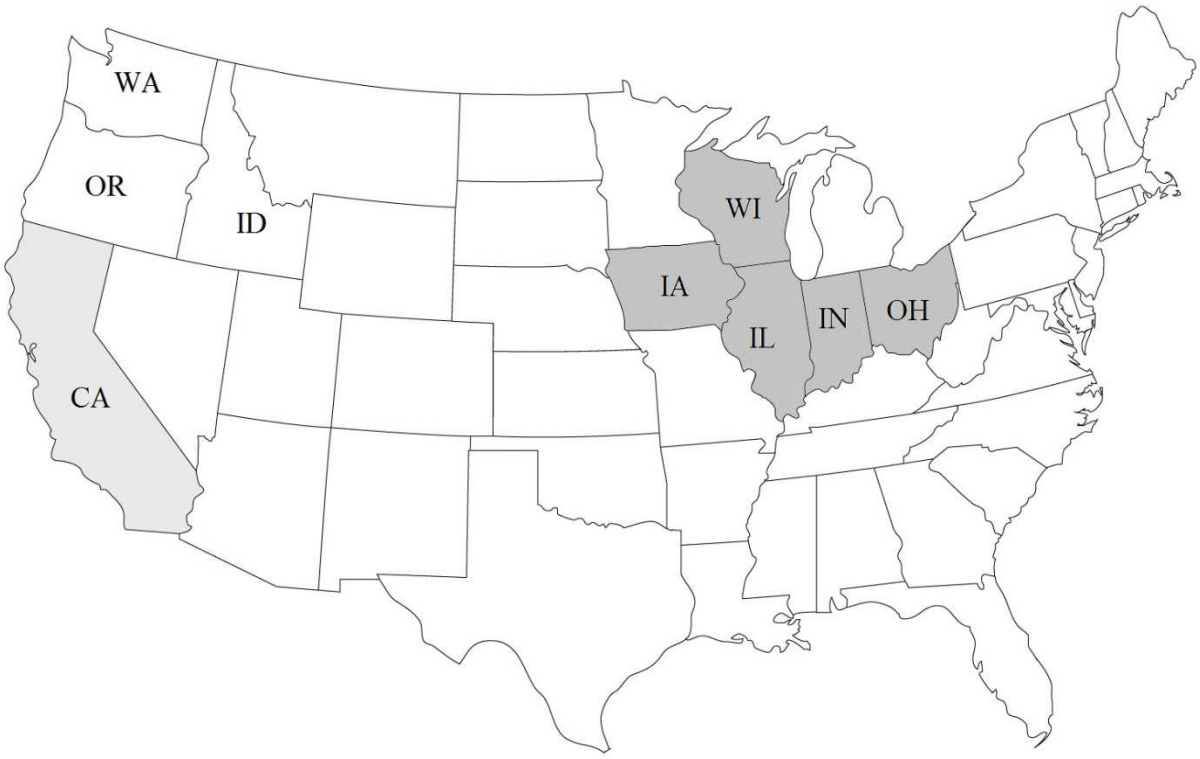

Figure 2: States selected from the NAWQA data export page (N = 2349) from 2000 to 2014 and grouped as Midwest (IA, IL, IN, OH, and WI), California (CA), and Northwest (WA, OR, ID). Peer reviewed studies were added to this database (N = 4).

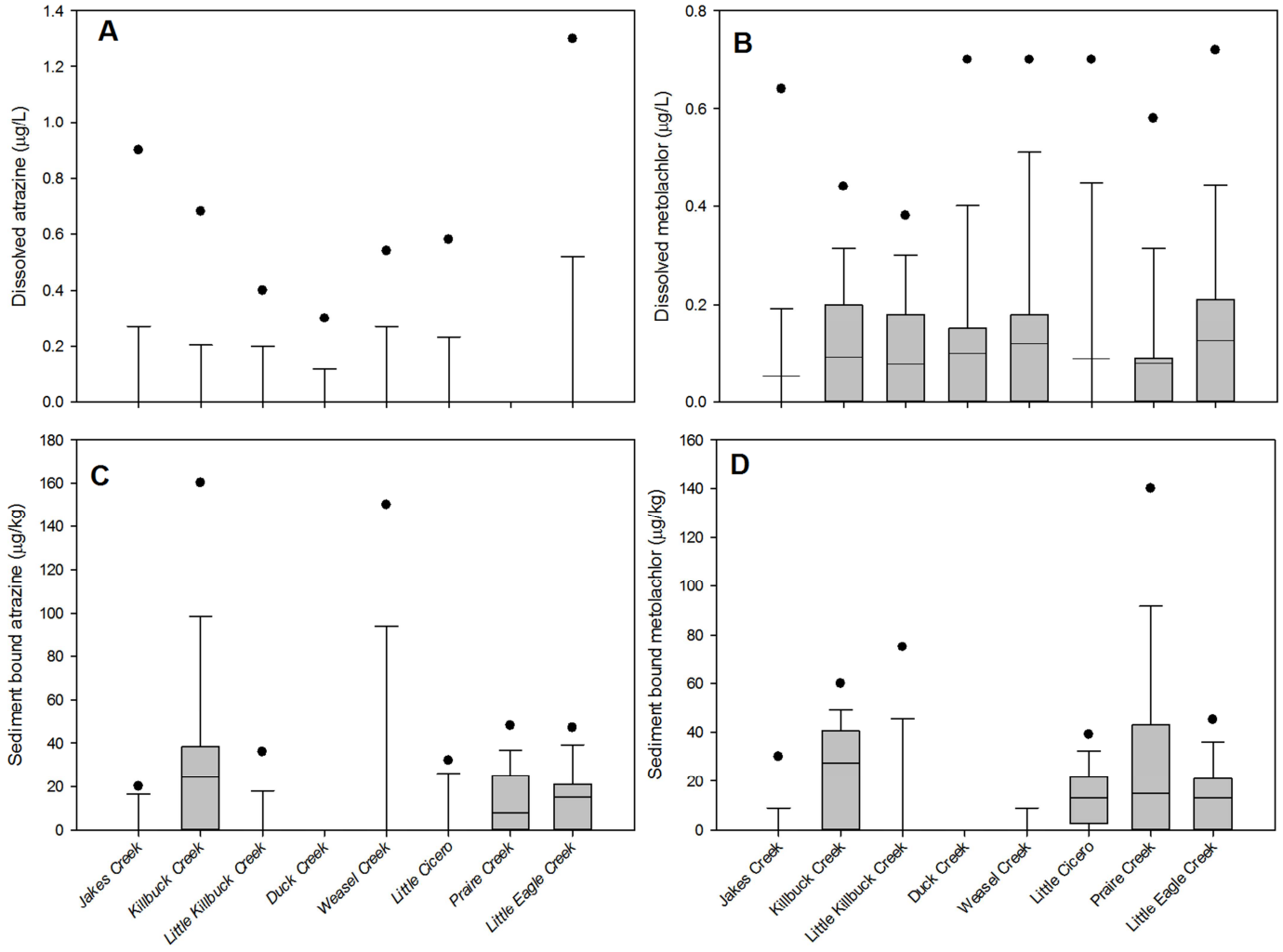

Figure 3: Boxplots of regional spatial variation in pesticide concentrations as A) Dissolved atrazine, B) Dissolved metolachlor, C) Sediment-bound atrazine and D) Sediment-bound metolachlor. Pesticide concentrations values were collected from eight sites sampled once monthly (October 2011 to September 2012).

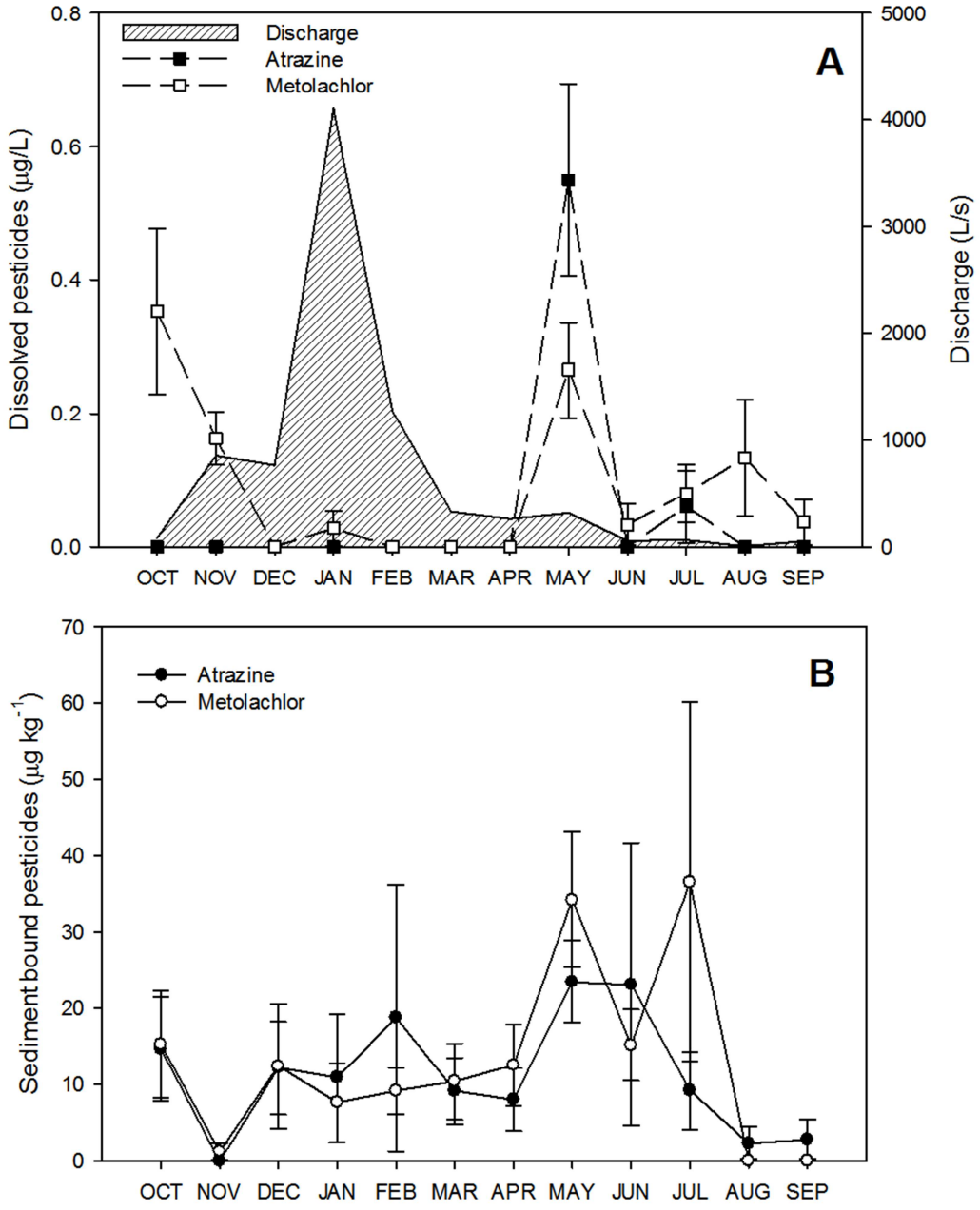

Figure 4: Regional temporal variation in pesticide concentrations as A) Dissolved concentrations and B) Sediment-bound oncentrations of atrazine and metolachlor. Stream discharge denoted as shaded area (panel A). Pesticide concentrations and discharge values are mean (± standard deviation) of eight sites sampled once monthly.

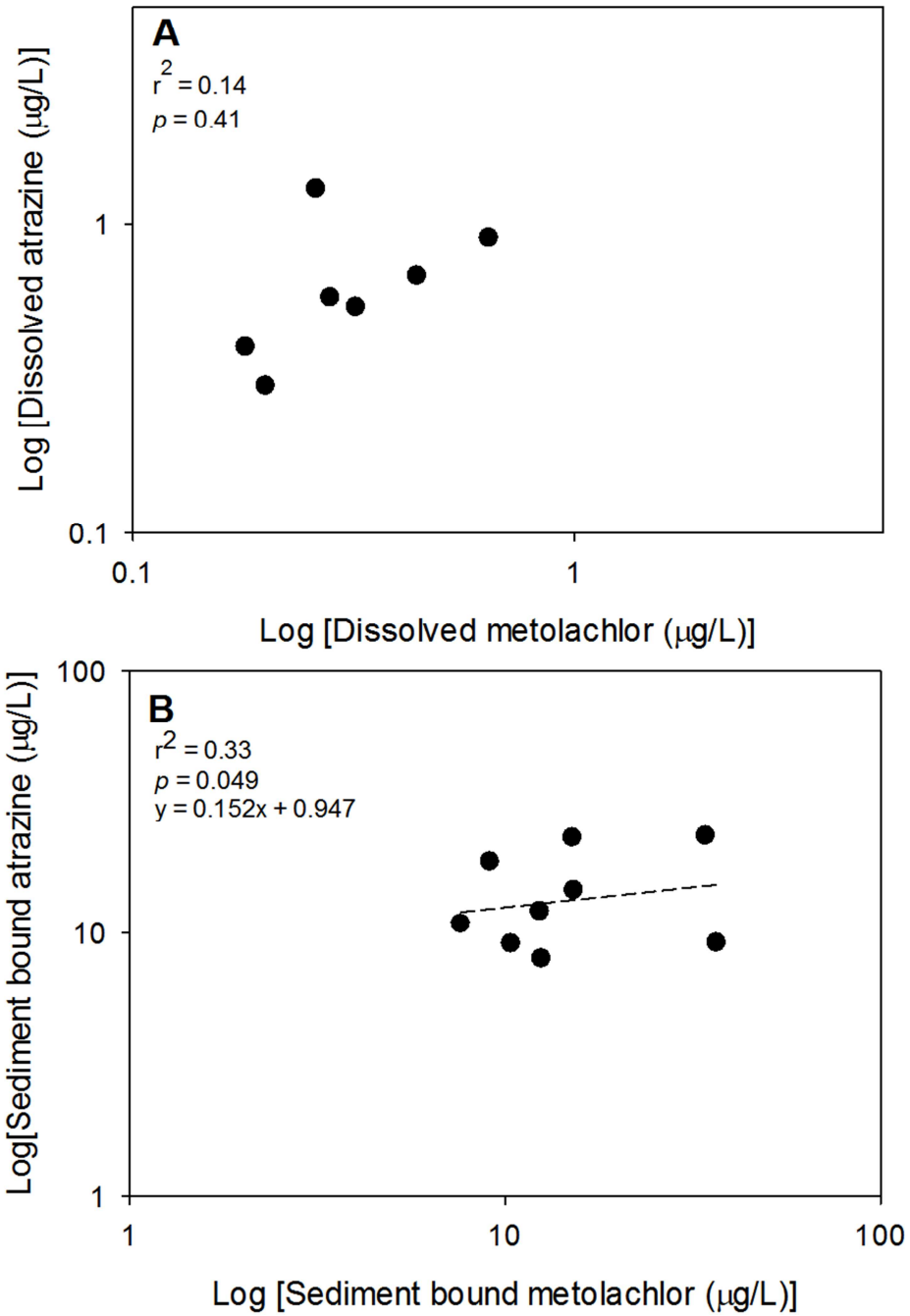

Figure 5: Linear regressions between atrazine and metolachlor concentrations. X-axis and Y-axis are in a Log10 scale. A) Dissolved concentrations and B) Sediment-bound concentrations of these pesticides. Atrazine and metolachlor concentrations values include all data collected from eight sites sampled once monthly.

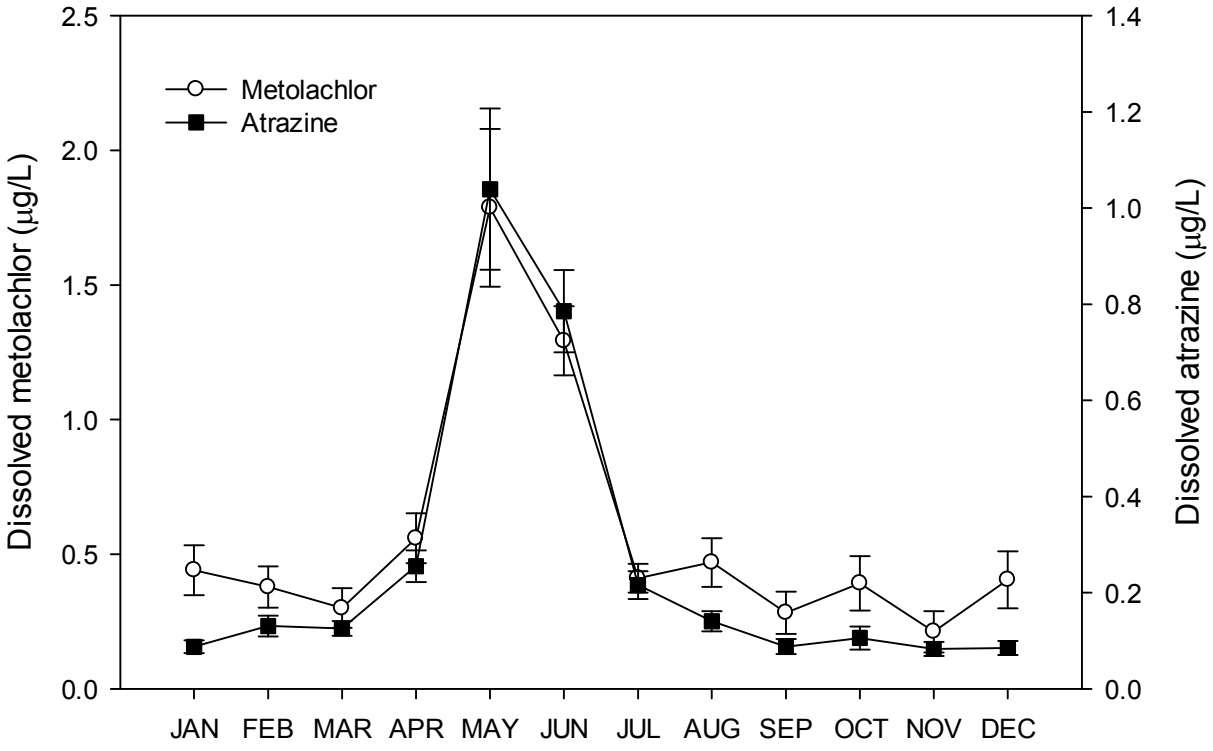

Figure 6: National peak concentrations of dissolved atrazine and metolachlor collected from the NAWQA data export page across nine states (IN, OH, IL, IA, WI, WA, OR, ID, and CA) from 2000 to 2014, and peer reviewed studies.

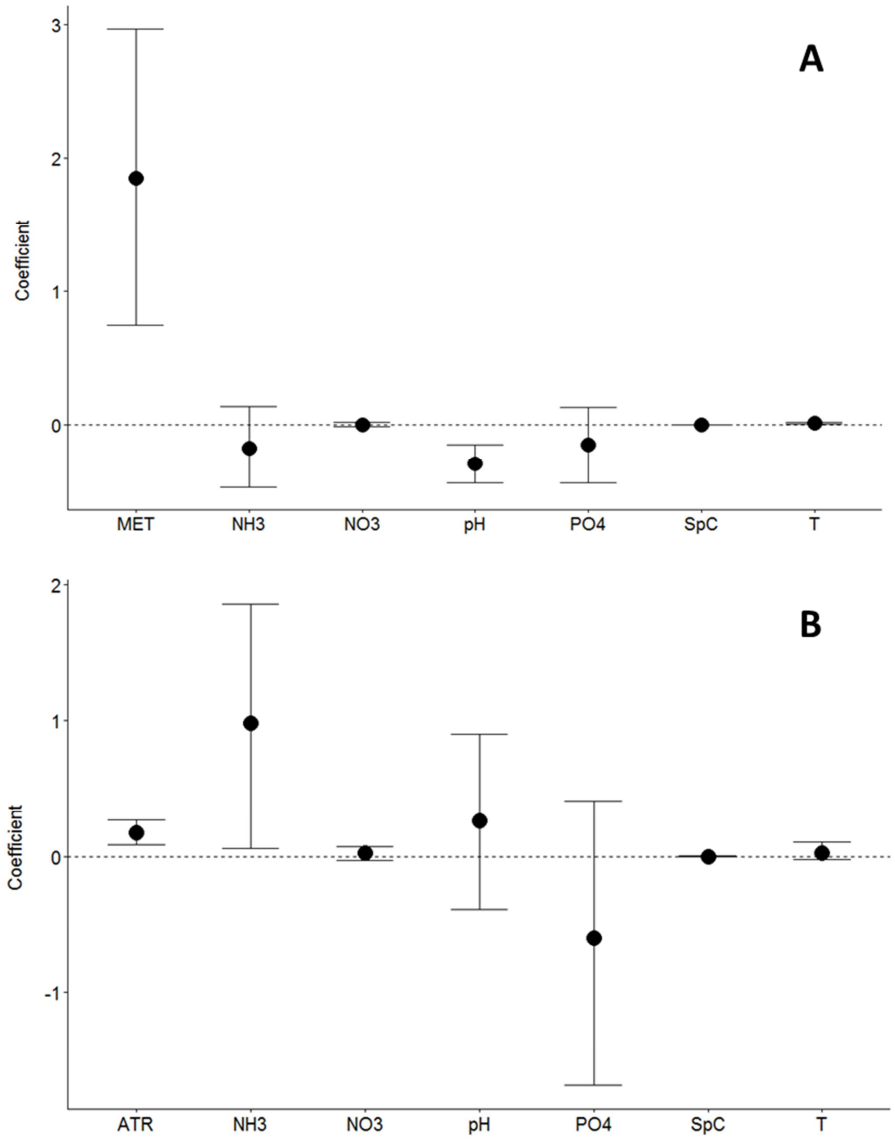

Figure 7: Population level distribution of coefficients in Model 2 for Atrazine (A) and Metolachlor (B). Black circles are medians of the posterior distribution and error bars represent 95% credible intervals. Bars that do not overlap zero represent significant effects of variables (Metolachlor, pH and temperature) on atrazine (A) significant effects of variables (Ammonia and atrazine) on metolachlor (B).

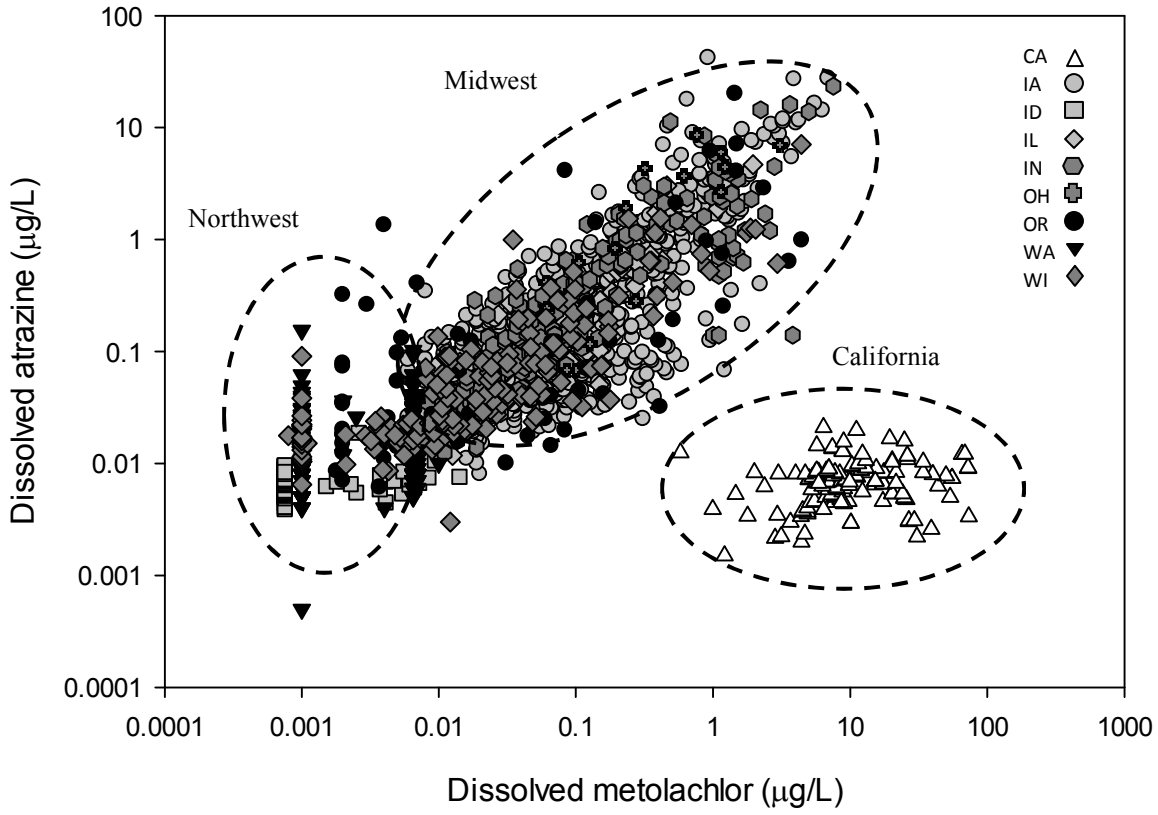

Figure 8: Dissolved metolachlor and atrazine concentrations (N = 2349) across nine states and grouped by Midwest (IN, OH, IL, IA, WI), California (CA), and Northwest (WA, OR, ID). Axes were log transformed.

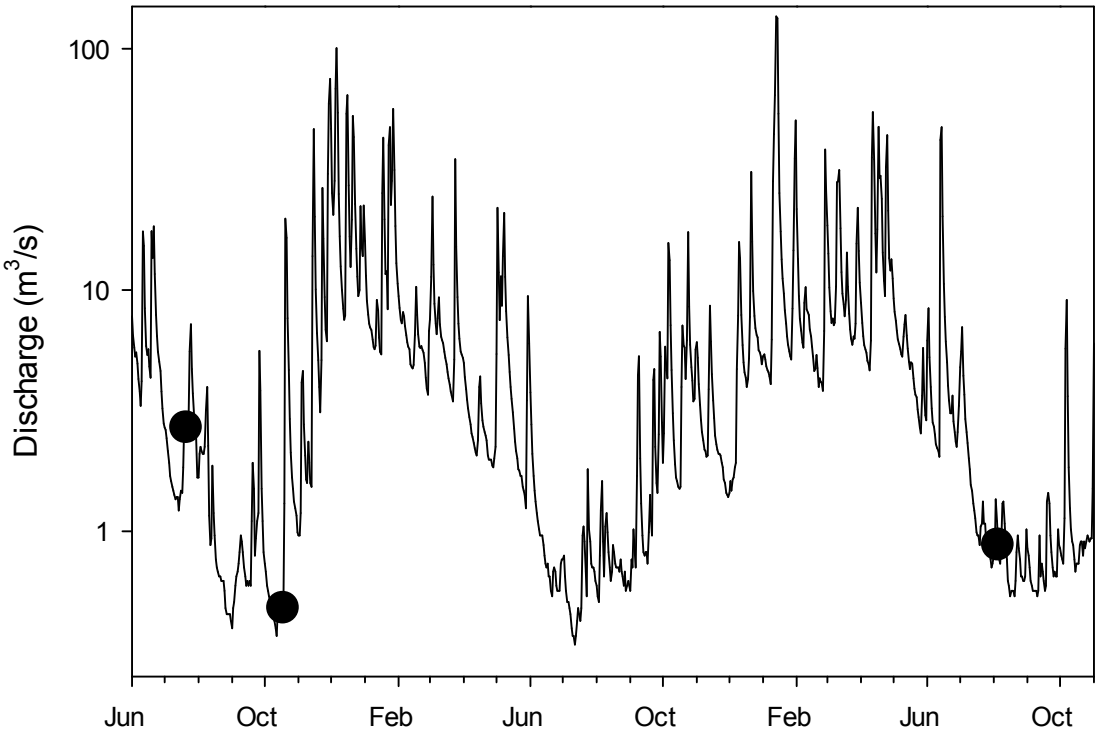

Figure 9: Hydrograph of the White River, Muncie IN (USGS 03347000) used as proxy for discharge in Jakes Creek. Hydrograph shows in bold circles the three enrichment events: July 21 2011, October 18 2011, and August 05 2013.

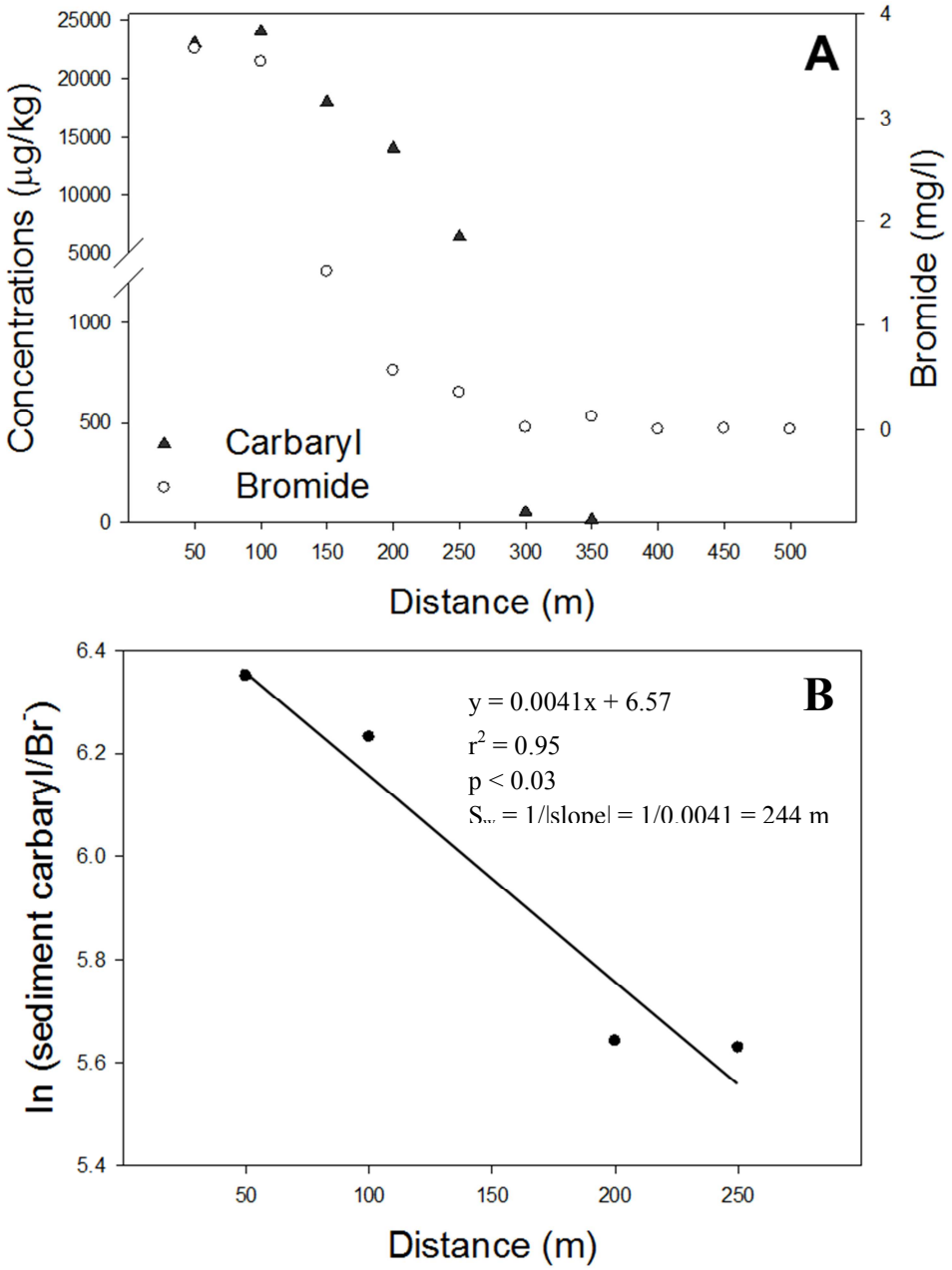

Figure 10: First enrichment (July 21 2011) showing A: Sediment-bound concentration of carbaryl (µg/kg) and dissolved concentration of bromide (mg/L); B: Bromide corrected concentrations of sediment-bound carbaryl.

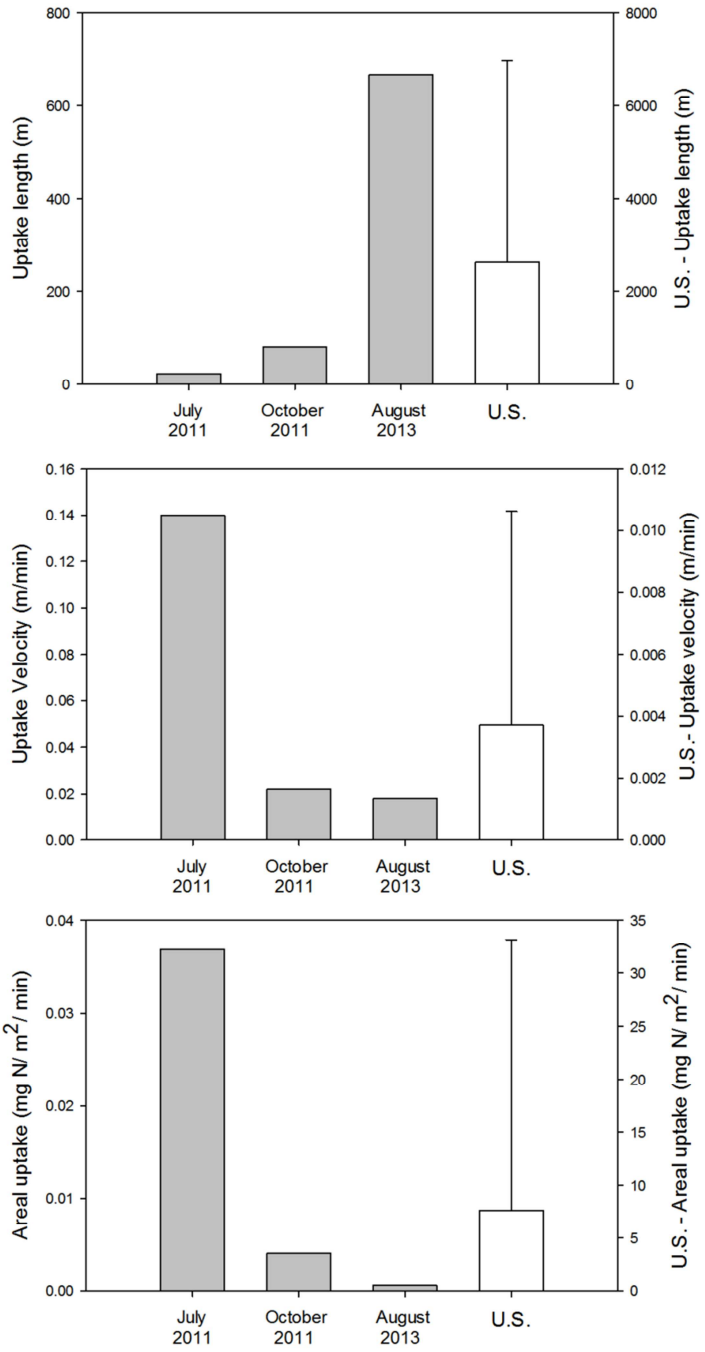

Figure 11: Comparison of nitrate uptake length (m), uptake velocity (m/min) and areal uptake (mg $N/m^2/min$) calculated for July 2011, October 2011, August 2013 enrichment events, and U.S. average of 23 agricultural streams across the U.S. and Puerto Rico (Mulholland et al. 2008).

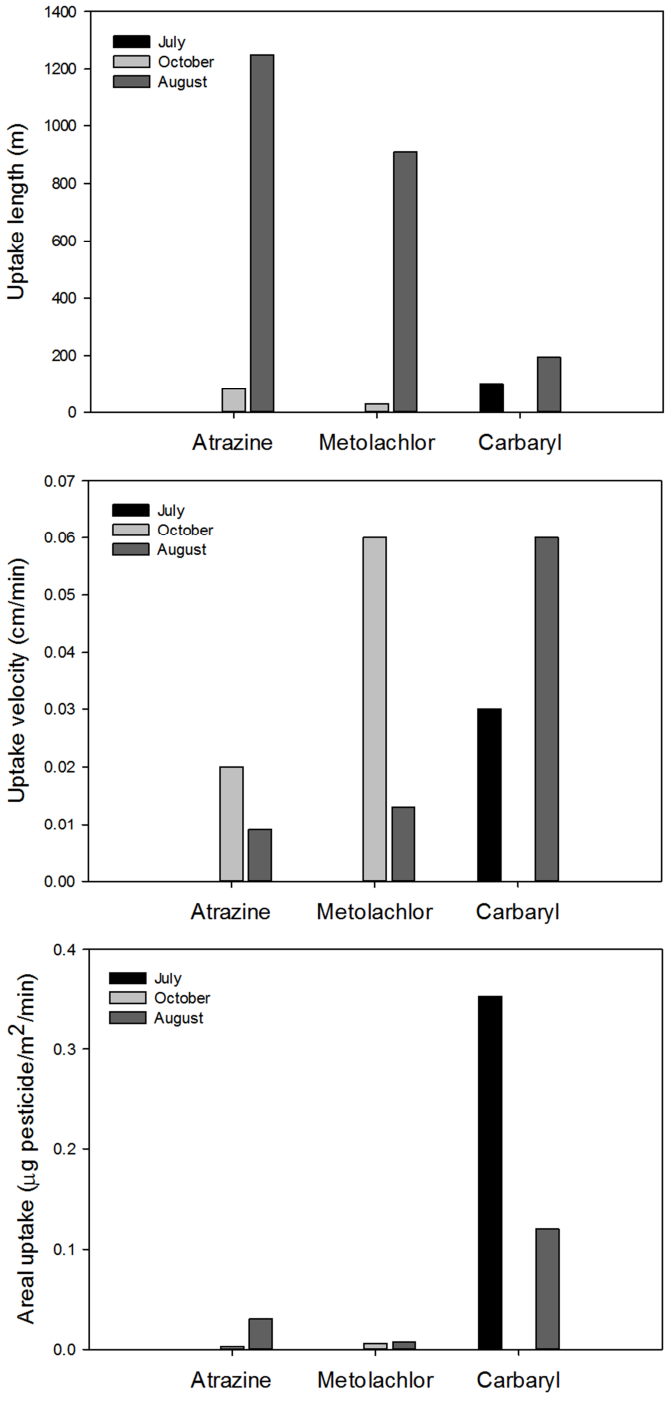

Figure 12: Uptake length (m), uptake velocity (cm/min), and areal uptake (µg pesticide/m^2/min) of dissolved concentrations of atrazine and metolachlor (October 2011 and August 2013), and carbaryl (July 2011 and August 2013) calculated for each enrichment event.

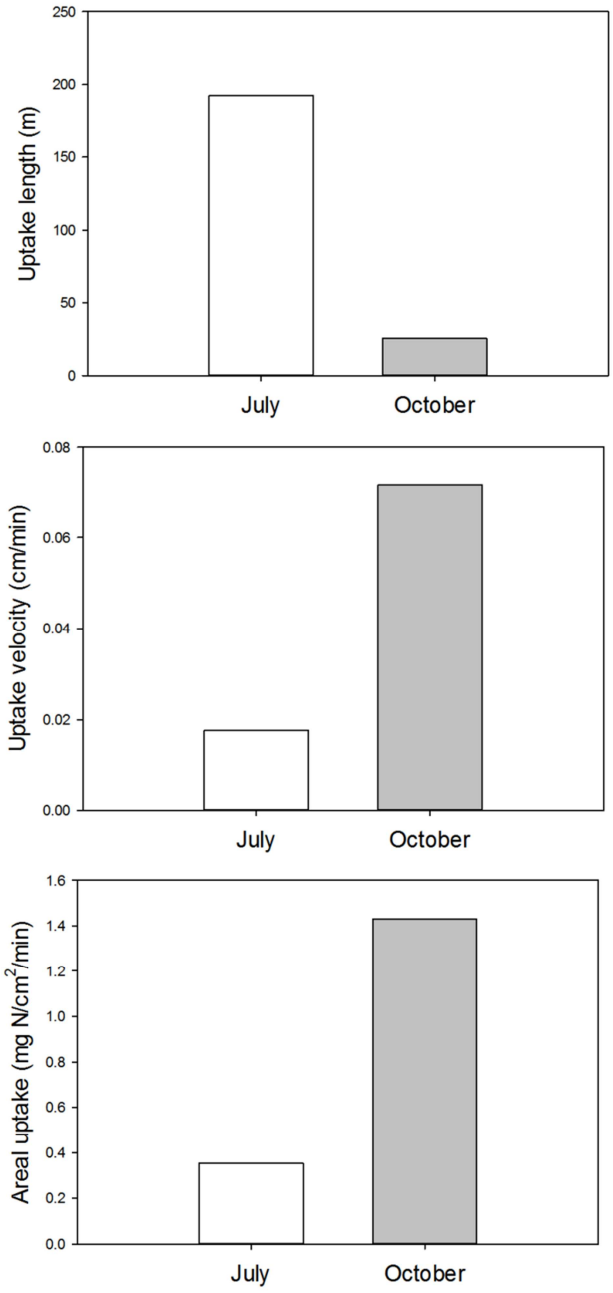

Figure 13: Uptake length (m), uptake velocity (m/min) and areal uptake (µg sediment-bound carbaryl/cm^2/min) of sediment-bound carbaryl from July 2011, and October 2011 enrichment events.

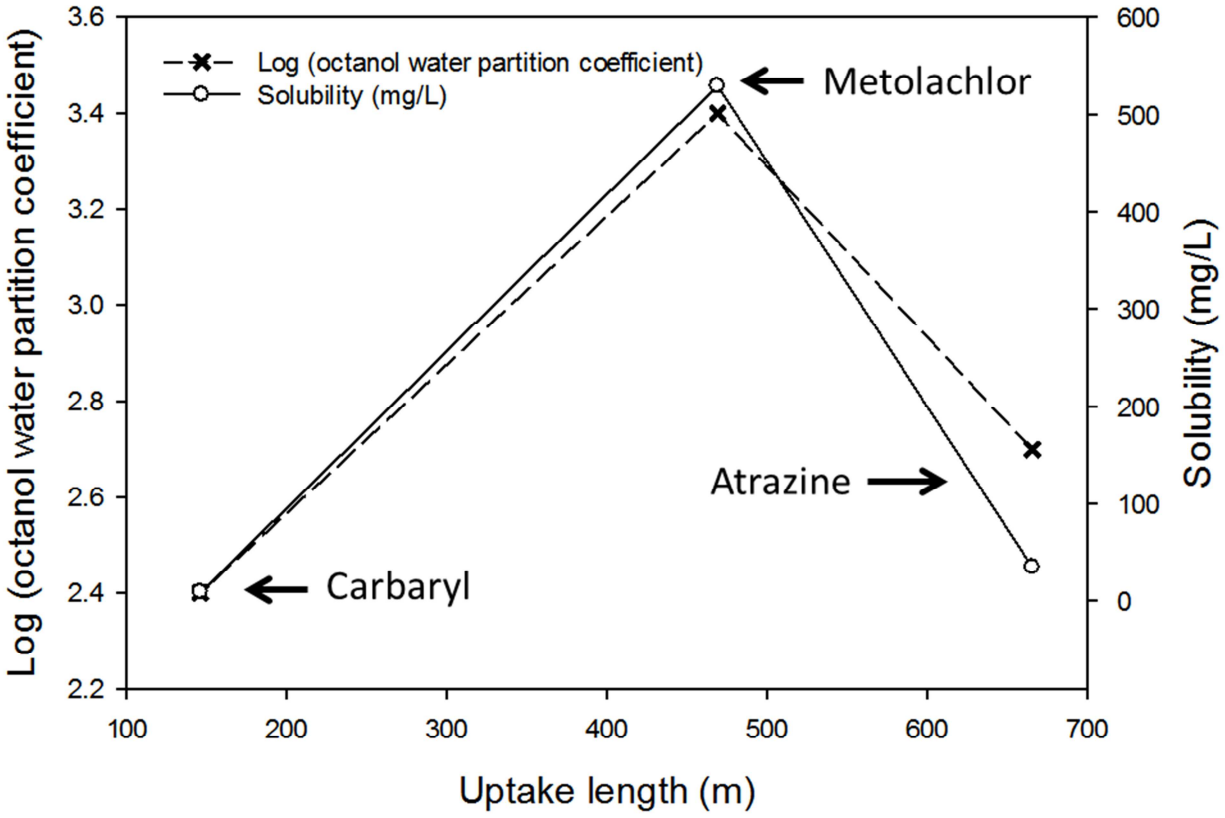

Figure 14: Relationship between atrazine, metolachlor, and carbaryl uptake lengths and their physicochemical properties (Log- octanol water partition coefficient and solubility).

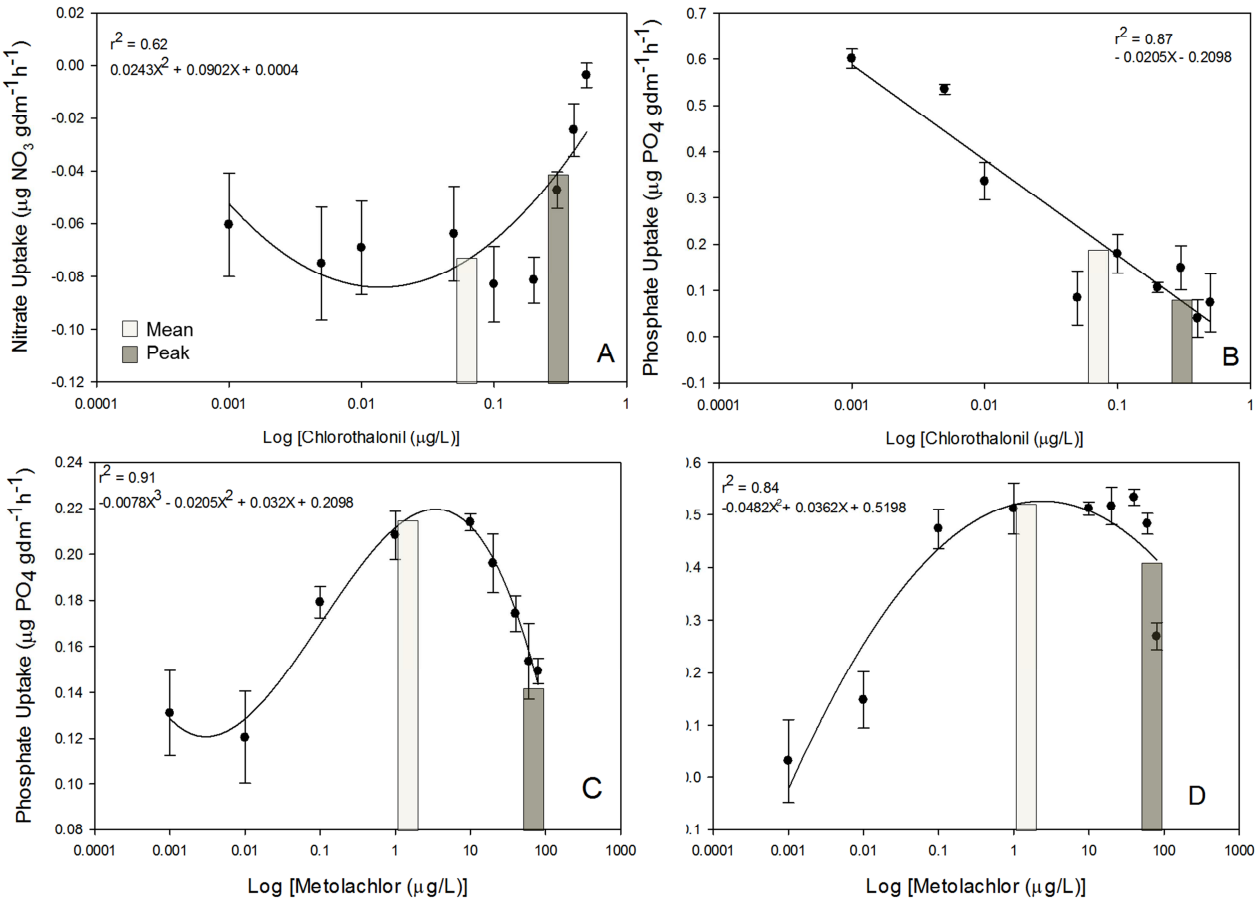

Figure 15: Nutrient uptake rates (mean +/- SE) response to pesticide concentrations after 24 h incubation (4 replicates, 10 treatments, N = 40). X-axis is in a Log10 scale. A: Nitrate uptake in response to chlorothalonil concentrations. B: Phosphate uptake rate response to chlorothalonil concentrations. C: Phosphate uptake rate response to metolachlor concentrations. D: Ammonium uptake rate response to metolachlor concentrations. Columns represent predicted uptake rates for each nutrient calculated at mean and peak concentrations of metolachlor and chlorothalonil measure in U.S. freshwaters (Table 10).

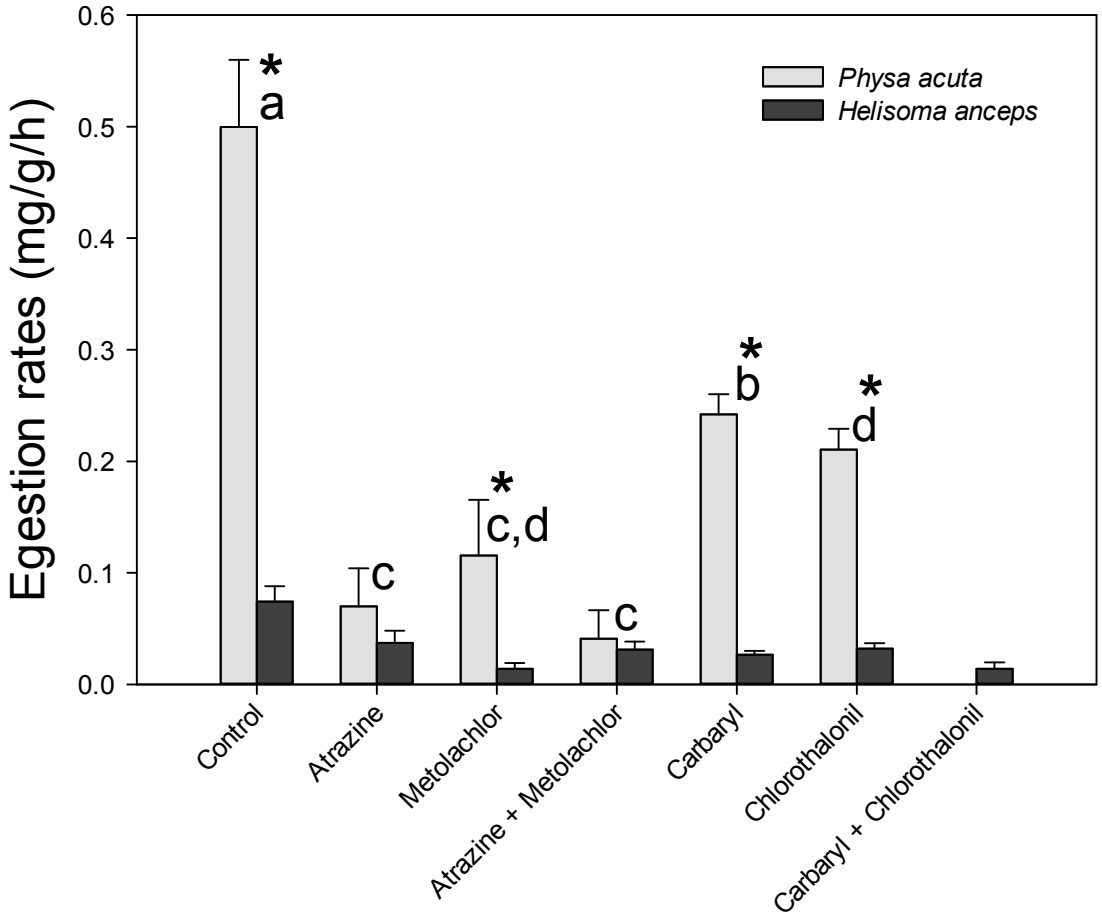

Figure 16: *Physa acuta* and *H. anceps* egestion rates (mean ± SE) exposed to atrazine, metolachlor, carbaryl, chlorothalonil, atrazine + metolachlor, and carbaryl + chlorothalonil concentrations. Different letters represent significant differences between treatments for *P. acuta*. (*) represents significant differences between egestion rates of *P. acuta* and *H. anceps*.

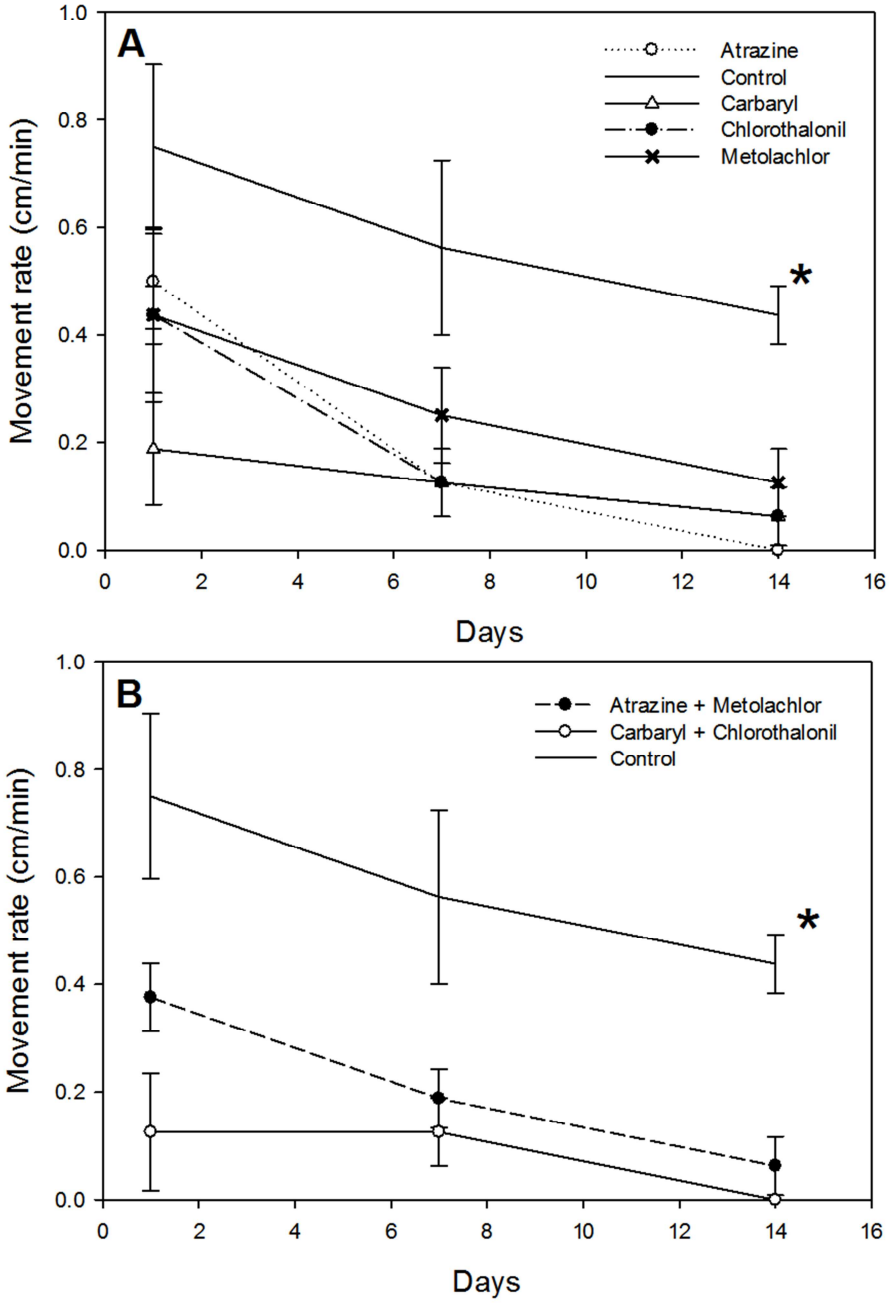

Figure 17: *H. anceps* movement rate (mean ± SE) after 24 hours, 1 week, and 2 weeks exposure to (A) individual atrazine, metolachlor, carbaryl, and chlorothalonil, and combined (B) atrazine + metolachlor and carbaryl + chlorothalonil. * Represents significant differences between control treatment and pesticide treatments ($p < 0.05$).

TABLES

Table 1: Eight streams sampled monthly from October 2011 to September 2012 (Dates sampled: 10-27-2011, 11-30-2011, 12-13-2011, 01-27-2012, 02-16-2012, 03-22-2012, 04-26-2012, 05-16-2012, 06-19-2012, 07-17-2012, 08-14-2012, and 09-30-2012).Soil type, mean annual precipitation, and mean annual air temperature were collected from the USDA - NRCS, Web Soil Survey. Hydrological Unit Codes (HUC) and size was determined from the USGS, Science in Your Watershed.

Stream	Coordinates		Watershed	HUC	Watershed size (km^2)	Soil type	Mean annual precipitation (mm)	Mean annual air temperature (C^0)
Weasel Creek	40.21483	-85.96737	Cicero Creek	512020106	582.8	Patton silty clay loam (Pn)	914 - 1066	9.4 - 11.1
Little Cicero Creek	40.19159	-86.10865	Cicero Creek	512020106	582.8	Shoals silt loam (Sh)	914 - 1066	9.4 - 11.1
Prairie Creek	40.23893	-86.1073	Cicero Creek	512020106	582.8	Pella silty clay loams (Ps)	914 - 1117	9.4 - 12.2
Duck Creek	40.21883	-85.86245	Duck Creek	512020105	272.0	Shoals silt loam (Sh)	914 - 1066	9.4 - 11.1
Little Eagle Creek	40.04186	-86.19752	Eagle Creek	512020111	419.6	Shoals silt loam (Sh)	914 - 1066	9.4 - 11.1
Killbuck Creek	40.25934	-85.51991	Killbuck Creek White River	512020103	1437.4	Sloam silt loam (SmsAH)	914 - 1092	8.9 - 12.2
Little Killbuck Creek	40.21916	-85.62607	Killbuck Creek White River	512020103	1437.4	Westland silty clay loam (Wd)	837 - 1066	8.3 - 11.7
Jakes Creek	40.24317	-85.46183	Killbuck Creek White River	512020103	1437.44	BellCreek silty clay loam (BdhAH)	914 - 1092	8.9 - 12.2

Table 2: EPA methods used for analytical analysis of atrazine and metolachlor in water and sediment samples collected at eight streams from October 2011 to September 2012. Method detection limits (MDL) for each EPA method.

Pesticide	Water		Sediment	
	EPA Method	**MDL (µg/L)**	**EPA Method**	**MDL (µg/L)**
Atrazine	525.2	0.078	525.2	14
Metolachlor	525.2	0.09	525.2	9

Table 3: Correlation coefficients for regional and national physicochemical parameters with non-metric multidimensional scaling (NMDS) axes. At the regional scale, discharge, temperature and dissolved oxygen (mg/L) was related to NMDS axis 1. Specific conductivity, salinity and total dissolved solids were correlated to NMDS axis 2. At the national scale, only one variable was correlated with each NMDS axes.

Parameters	NMDS1	NMDS2
Regional		
Discharge (L/s)	***0.92***	-0.10
Water Temperature (°C)	***-0.58***	-0.23
DO (mg/L)	***0.59***	0.31
Specific Conductivity (µS/cm)	-0.29	***-0.91***
Salinity (ppt)	-0.30	***-0.91***
Total dissolved solids (g/L)	-0.29	***-0.91***
National		
Water Temperature (°C)	0.11	***0.58***
Specific Conductivity (µS/cm)	***-0.77***	0.33

Table 4: Physicochemical characteristics, nutrients and pesticide concentrations collected in the UWRW of central Indiana. Physicochemical parameters, water and sediment samples were collected across eight different streams from October 2011 to September 2012 (N = 12). ATR: Atrazine, MET: Metolachlor, T: Temperature, SpC: Specific conductivity, DO: Dissolved oxygen, (*): pesticides below detection limit. Standard deviation noted in parentheses.

Date	NO_3 (mg/L)	PO_4 (mg/L)	NH_4 (µg/L)	Total Organic Carbon (mg/L)	ATR (µg/L)	MET (µg/L)	Sediment ATR (µg/kg)	Sediment MET (µg/kg)	Discharge (L/s)	Water T (C°)	SpC (µS/cm)	Salinity (ppt)	Total Dissolved Solids (g/L)	pH	DO (mg/L)
OCT	1.6 (1.6)	0 (0)	0.17 (0)	120.9 (14)	*	0.4 (0.4)	14.6 (19.3)	15.3 (19.8)	80 (74)	11.6 (0.6)	680 (101)	0.35 (0.1)	0.43 (0.1)	7.88 (0.2)	6.0 (1.1)
NOV	10.1 (7.1)	0.1 (0.1)	0.17 (0)	56.2 (5.2)	*	0.2 (0.1)	*	1.3 (3.3)	855 (729)	4.6 (1)	259 (39)	0.12 (0)	0.16 (0)	7.53 (0.3)	10.9 (0.7)
DEC	8.8 (5.3)	0 (0)	0.17 (0)	98.4 (9.1)	*	*	12.1 (17.2)	12.4 (23)	763 (681)	5.3 (0.7)	565 (36)	0.29 (0)	0.36 (0)	7.37 (0.5)	10.9 (1.4)
JAN	10.6 (5.3)	0 (0)	0.17 (0)	75.8 (12.4)	*	0.03 (0.1)	10.9 (23.6)	7.6 (14.6)	4102 (3730)	3.8 (0.7)	400 (64)	0.2 (0)	0.26 (0)	7.76 (0.2)	11.5 (0.9)
FEB	16.3 (9.6)	7.1 (3.9)	0.16 (0)	77.6 (10.6)	*	*	18.8 (49.6)	9.1 (8.6)	1272 (1182)	5.4 (0.4)	574 (77)	0.29 (0)	0.37 (0.1)	7.81 (0.1)	11.3 (1.4)
MAR	7.6 (5.8)	3.2 (2)	0.16 (0)	111.3 (10)	*	*	9.1 (12.4)	10.4 (14.1)	325 (264)	16.1 (1)	588 (44)	0.3 (0)	0.38 (0)	7.58 (0.3)	7.5 (1.7)
APR	5.4 (4.3)	1.3 (1.8)	0.17 (0)	88.7 (13.9)	*	*	8 (11.5)	12.5 (15.1)	258 (209)	13.4 (0.5)	610 (58)	0.31 (0)	0.39 (0)	7.84 (0.1)	7.2 (1.1)
MAY	6.9 (4.3)	0.1 (0.2)	0.16 (0)	108.7 (11.8)	0.6 (0.4)	0.3 (0.2)	23.5 (15.3)	34.3 (25.1)	314 (259)	16.8 (0.9)	521 (35)	0.27 (0)	0.33 (0)	7.98 (0.1)	7.1 (1.2)
JUN	0.8 (0.8)	0.4 (0.4)	0.16 (0)	122.6 (17.1)	*	0.03 (0.1)	23.1 (52.4)	15.1 (13.2)	58 (84)	22 (1.4)	588 (120)	0.3 (0.1)	0.38 (0.1)	7.8 (0.2)	3.8 (1.2)
JUL	0.7 (1.1)	0.7 (0.5)	0.18 (0)	109.9 (18.9)	0.1 (0.1)	0.1 (0.1)	9.2 (11.3)	36.6 (52.6)	62 (82)	25.7 (0.4)	532 (157)	0.27 (0.1)	0.34 (0.1)	7.9 (0.1)	2.8 (0.3)
AUG	5.1 (5.9)	0.1 (0.2)	0.18 (0)	4.2 (1.0)	*	0.1 (0.2)	2.3 (5.2)	*	6 (10)	20.6 (0.7)	453 (168)	0.23 (0.1)	0.29 (0.1)	8.3 (0.1)	5.4 (0.6)
SEP	7.5 (8.4)	0.1 (0.1)	0.18 (0)	6.7 (1.9)	*	0.04 (0.1)	2.8 (6.3)	*	53 (6)	14.4 (0.4)	606 (157)	0.31 (0.1)	0.37 (0.1)	8.29 (0.1)	7.3 (0.9)

Table 5: Physicochemical stream parameters, nutrients, and atrazine and metolachlor concentration collected from the NAWQA data export page and peer reviewed studies (N = 2349) across nine states from 2010 to 2014. Values are means values with standard deviations noted in parentheses.

State	NH_3 (µg/L)	NO_3 (mg/L)	PO_4 (mg/L)	Water Temperature (C°)	Specific Conductivity (µS/cm)	pH	Metolachlor (µg/L)	Atrazine (µg/L)
CA	0.1(0.4)	3.1(1.8)	0.6(0.4)	15.8(5.1)	741(315)	7.9(0.4)	14.75(15.24)	0.007(0.003)
IA	0.1(0.2)	5(3.01)	0.1(0.1)	13.4(9.3)	576(167)	8.1(0.4)	0.19(0.5)	0.57(2.15)
ID	0.1(0.1)	1.8(0.6)	0(0.01)	10.9(4.1)	627(144)	8.1(0.3)	0.002(0.003)	0.007(0.002)
IL	0.1(0.05)	3.3(2.6)	0.3(0.5)	15.2(8.5)	717(470)	7.9(0.3)	0.13(0.26)	0.37(0.77)
IN	0(0.1)	2.9(3.9)	0.1(0.1)	14(8.3)	496(154)	8.1(0.6)	0.31(0.75)	0.76(2.3)
OH	0(0.04)	2.7(2.4)	0.1(0.05)	16.7(8.6)	598(215)	8.1(0.3)	0.51(0.72)	2.2(2.63)
OR	0.2(0.5)	3.1(4.8)	0.2(0.2)	13.8(4.8)	214(147)	7(0.3)	0.33(0.78)	0.81(2.66)
WA	0.1(0.1)	13.3(7.6)	0.4(0.1)	14.8(3.3)	415(161)	8.1(0.2)	0.005(0.003)	0.02(0.02)
WI	0.1(0.3)	1.9(1.9)	0.1(0.1)	11.7(8.8)	1325(790)	7.9(0.3)	0.13(0.47)	0.18(0.59)

Table 6: EPA methods used for analytical analysis of atrazine, metolachlor, and carbaryl in water and sediment samples collected at each enrichment event in Jakes Creek (N = 3). Method detection limits (MDL) for each EPA method.

Pesticide	Water		Sediment	
	EPA Method	**MDL (µg/L)**	**EPA Method**	**MDL (µg/L)**
Atrazine	525.2	0.078	525.2	14
Metolachlor	525.2	0.09	525.2	9
Carbaryl	531.1	2	3550C	40

Table 7: Stream physicochemical parameters collected from 10 sampling stations at Jakes Creek in July and October 2011, and August 2013. Standard deviation noted in parentheses. TDS: Total dissolved solids.

	Temperature (C°)	Specific conductivity (µS/cm)	Salinity (ppt)	TDS (g/L)	pH	Dissolved Oxygen (%)	Discharge (L/s)
Jul-11	23.7 (3.9)	606.5 (180.4)	0.3 (0.1)	0.4 (0.1)	6.8 (0.2)	54.2 (12.1)	8.1 (1.2)
Oct-11	19.8 (2.9)	707.1 (112.0)	0.3 (0.1)	0.4 (0.1)	7.5 (0.2)	51.1 (6.6)	6.0 (13.9)
Aug-13	21.9 (2.7)	629.1 (102.8)	0.3 (0.1)	0.4 (0.1)	7.7 (0.2)	65.0 (7.5)	35.1 (8.8)

Table 8: Uptake length: S_w (m), uptake velocity: V_f (cm/min), and areal uptake: U (mg N/cm^2/min) of dissolved samples of atrazine, metolachlor, and carbaryl, as well as sediment-bound carbaryl samples collected at each enrichment event in Jakes Creek (N = 3).

	Atrazine			**Metolachlor**			**Carbaryl**			**Carbaryl (sediment)**		
	Sw	**V_f**	**U**	**Sw**	**V_f**	**U**	**Sw**	**V_f**	**U**	**Sw**	**V_f**	**U**
Jul-11	-	-	-	-	-	-	100	0.03	0.352	192	0.02	0.034
Oct-11	82	0.02	0.003	29.6	0.06	0.006	-	-	-	25.5	0.07	1.43
Aug-13	1250	0.009	0.03	909	0.013	0.007	193.3	0.06	0.12	-	-	-
Mean	666	0.015	0.017	469.3	0.037	0.007	146.7	0.045	0.236	108.8	0.045	0.732

Table 9: Detection frequency and concentrations of atrazine, metolachlor, carbaryl, and chlorothalonil in U.S. freshwaters: Detection frequency and concentrations of atrazine, metolachlor, carbaryl, and chlorothalonil in U.S. freshwaters. Detection frequency was estimated throughout the U.S. across 50 basins (33 agricultural, 10 urban and 7 mixed); mean and maximum concentrations correspond to 83 agricultural streams. Annual mean detection frequencies for each compound at each site provide the proportion of water samples that have detectable levels of pesticides for a year period. *Chlorothalonil was detected at concentrations of 290 µg/L in runoff near golf courses.

Compound	Detection frequency (%)	Mean concentration (µg/L)	Maximum concentration (µg/L)	Literature cited
Atrazine *(Herbicide)*	78.1	2.4	201	(15, 16, 17)
Metolachlor *(Herbicide)*	71.1	1.2	77.6	(15, 16, 17)
Carbaryl *(Insecticide)*	18.1	0.013	4.78	(15, 16, 17)
Chlorothalonil *(Fungicide)*	0.033	<0.07	0.29 (290*)	(7, 14, 17)

Table 10: Toxicity and Octanol-water partition coefficient of atrazine, metolachlor, carbaryl and chlorothalonil to daphnids, green algae, and humans. Toxicity of atrazine, metolachlor, carbaryl and chlorothalonil in mg/L to daphnids and green algae, and mg/kg of body weight (bw) per day (d) to humans. No observed effect concentrations (NOEC) for daphnids and green algae were calculated by chronic tests of 21 days and 96 hours, respectively. Acceptable daily intake (ADI).* Half maximal effective concentration (EC50) of metolachlor on growth after 72 hours (19).

Compound	**Daphnids (mg/L)**	**Green algae (mg/L)**	**Humans (mg/kg/bw/d)**	**Octanol-Water partition coefficient**
	NOEC	**NOEC**	**ADI**	**Log K_{ow}**
Atrazine	0.25	0.1	0.02	2.7
Metolachlor	0.7	57.1*	0.1	3.4
Carbaryl	0.25	-	0.0075	2.4
Chlorothalonil	0.009	0.033	0.015	2.9

Table 11: Uptake rates for nitrate, phosphate, and ammonium in response to pesticides exposure. Uptake rates for nitrate, phosphate, and ammonium (μg gdm^{-1} h^{-1}) across pesticide and control (no pesticide) treatments. Range noted in parentheses. Mean uptake rate was calculated for each nutrient across pesticides.

Pesticide	**Nitrate (μg gdm^{-1} h^{-1})**	**Phosphate (μg gdm^{-1} h^{-1})**	**Ammonium (μg gdm^{-1} h^{-1})**
Atrazine	-25.88 (-45.2 - 4.30)	126.93 (83.1 - 169)	0.36 (0.23 - 0.45)
Metolachlor	-21.34 (-40.9 - 2.10)	169.54 (120 - 214)	0.39 (0.03- 0.53)
Carbaryl	-25.13 (-56.6 - 8.80)	192.11 (37.9 - 260)	0.31 (0.13- 0.53)
Chlorothalonil	-56.54 (-83.1 - -3.7))	233.67 (39.8 - 601)	0.28 (0.13 - 0.47)
Mean uptake rate	**-32.22**	**180.56**	**0.34**
Control	-1.58 (-1.62 - -1.49)	1.34 (7.03E-05 - 4.4)	0.03 (0.01 - 0.04)

Table 12: Mean and maximum pesticide concentrations reported in U.S waterways (EPA 1987; Kolpin et al. 1998; Larson et al. 1999; Relyea and Mills, 2001; Gilliom et al. 2006; McMahon et al. 2012). Treatment concentrations were comparable to peak concentrations detected throughout the U.S.

Compound	**Mean concentration (µg/L)**	**Maximum concentration (µg/L)**	**Treatment concentration (µg/L)**
Atrazine	2.4	201	200
Metolachlor	1.2	77.6	100
Carbaryl	0.013	90	100
Chlorothalonil	<0.07	290	100
Control	-	-	0